

# The Effects of Age and Sex of Megakaryocyte Secreted Factors on Endothelial Cell Growth and Function

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**INTRODUCTION:** With an aging population, the risk of fractures and compromised healing increases. Angiogenesis and vasculogenesis are impaired with aging. Vascularization at the callus plays a significant role in bone healing, and we have previously shown the important role of megakaryocytes (MKs) in regulating bone healing. Notably, MK-derived conditioned media (CM) contains factors known to promote angiogenesis. Whether differences exist with aging and/or based on sex was the primary focus of this study.

**METHODS:** Here, we examined the effects of CM secreted from MKs derived from young (3-4-month-old) and aged (22-24-month-old) male and female C57BL/6J mice on bone marrow endothelial cell (BMEC) growth and function. Specifically, BMEC proliferation, vessel-like formation, wound/transwell migration, and RNA expression were examined. The Indiana University School of Medicine Institutional Animal Care and Use Committee approved all described studies.

**RESULTS:** Both young and aged female MK CM resulted in a >65% increase in BMEC proliferation ( $p<0.001$  and  $p<0.05$ , respectively). In addition, female MK CM, regardless of age, improved all four parameters of vessel-like formation by >115% ( $p<0.05$ ). These parameters were the number of vessel-like structures formed, the structures' collective lengths, the number of nodes formed, and the number of meshes formed. Likewise, young male MK CM increased vessel-like formation in all parameters by more than 160% ( $p<0.001$ ). Although aged male MK CM resulted in higher vessel-like formation parameters, including significant >150% increases in the formation of nodes and meshes, 62% fewer vessel-like structures formed compared to that observed with young male MK CM treatment ( $p<0.05$ ). Additionally, aged MK CM, irrespective of sex, improved transwell migration by over 2500% ( $p<0.01$  in females, ns for males). On the other hand, aged female and male MK CM inhibited wound closure by 46% and 17%, respectively ( $p<0.05$ ). Treatment of BMECs with MK CM significantly altered the expression of several genes including PDGFRB, CXCR2, CD36, and CD74, not only relative to controls, but also between sexes and ages.

**DISCUSSION:** Female MK CM improved BMEC proliferation and increased all vessel-like structure parameters measured irrespective of age. Young and aged male MK CM increased nodes and meshes, while young MK CM alone increased the number and length of vessel-like structures formed. BMECs treated with aged male and female MK CM exhibit impaired wound closure, but female aged MK CM significantly increased BMEC transwell migration. MK CM, regardless of age or sex, increased expression of PDGFRB and CXCR2 and decreased expression of CD36 and CD74 to an extent.

**SIGNIFICANCE/CLINICAL RELEVANCE:** MK CM improves many aspects of BMEC growth and function, although some aspects of migration are inhibited by aged MK CM. Further testing to identify the mechanisms responsible for these age-associated differences may allow for novel treatment strategies to improve MK-mediated angiogenesis, vasculogenesis, and bone healing, particularly within the aging population.

## IMAGES AND TABLES:

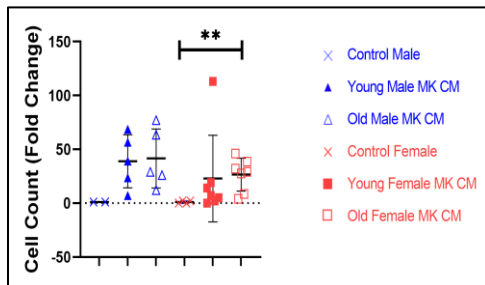


Figure 1: Changes in the number of BMECs migrated across transwell membrane in transwell assay.

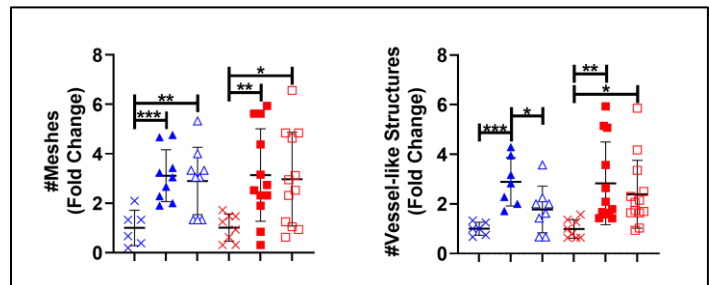


Figure 2: Changes in the number of nodes and meshes of BMECs in vessel-like formation assay.

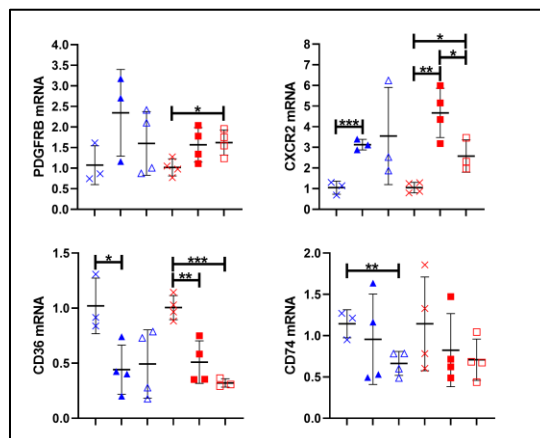


Figure 3: Changes in RNA expression of PDGFRB, CXCR2, CD36, and CD74 in BMECs.