Reprogramming Method for Induced Pluripotent Stem (iPS) Cells Derived from Mouse Skin Fibroblasts and Perspectives for Bone Regenerative Therapy

Mayu Kurihara[1, 3], Simon Kwoon-Ho Chow[1], Qi Gao[1], Chao Ma[1], Takahiro Igei[1], Ryota Hirose[1], Yosuke Susuki[1], Issei Shinohara[1], Masatoshi Murayama[1], Max L. Lee[1], Stuart B. Goodman[1, 2]
[1]Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, CA,
[2]Department of Bioengineering, Stanford University, Stanford, CA
[3]Division of Oral Maxillofacial Surgery, Department of Dentistry and Oral Surgery, Keio University School of Medicine
Email of Presenting Author: marcy5@stanford.edu

Disclosures: All authors have no potential conflict of interest

INTRODUCTION:
Research on induced pluripotent stem cells (iPSCs) has undergone remarkable progress in recent years. Despite concerns about tumorigenic risk, iPSCs have attracted significant attention in the field of regenerative medicine. In bone regeneration, however, iPSC-based studies remain relatively limited.
Bone regeneration is known to be impaired with aging, and several mechanisms have been identified. Aging reduces the number and regenerative potential of skeletal stem and progenitor cells, while also impairing the function of mesenchymal stem cells (MSCs). Furthermore, inflammaging—chronic low-grade inflammation associated with aging—exacerbates tissue repair dysfunction through senescence-associated secretory phenotype (SASP) factors. Age-related deterioration of periosteal MSCs further compromises the supply of reparative cells, and structural changes in bone matrix, such as collagen cross-linking and stiffening, reduce tissue flexibility and delay fracture healing. Clinically, this manifests as slower and poorer bone repair in elderly individuals compared with younger patients, underscoring the urgent need for alternative therapeutic strategies.
MSC-based therapy has attracted considerable interest and shown promise in preclinical and early clinical studies. However, harvesting MSCs from bone marrow is invasive and difficult to scale. In parallel, iPSCs are being explored as a less invasive and more versatile cell source, but their application in bone regeneration is still at an early stage. To address this challenge, we attempted to establish iPSCs from aged mouse skin fibroblasts as a model. Although mice and humans are distinct, our ultimate goal is to explore iPSC-derived MSCs (iMSCs) as a less invasive and potentially more effective cell source, thereby laying the groundwork for future clinical applications in bone regenerative therapy.

METHODS:
The experimental methods are shown in **Figure 1**. Skin specimens were harvested freshly from four (n=4) 18-24 months old male C57BL/6 mice and cultured for x number of days for primary fibroblasts and cells at passage x were reprogrammed using the STEMCCA™ lentiviral vector kit (MilliporeSigma, Burlington, MA, USA) according to the manufacturers protocol. Confirmation of reprogramming is confirmed by immunocytochemistry (ICC) for pluripotency factors of Oct4+, Sox2+, and DPPA-2+, SSSEA-1+ after passage5.

RESULTS:
The mean reprogramming efficiency was 50.08% (**Figure 2**). While colony formation and colony pick-up were generally successful, most clones failed to be maintained up to passage 5 due to proliferation arrest and poor adhesion, resulting in cell detachment. The morphology of the four established iPSC lines is shown in **Figure 3**. Each line exhibited differences in colony morphology and proliferation rate (**Figure 3a**). For the established iPSC clones, vector integration was confirmed by Oct4 and Sox2 ICC, and pluripotency was further validated using DPPA-2 and SSEA-1 markers (**Figure 3b**).

DISCUSSION:
We established iPSCs from skin fibroblasts of aged mice. While the reprogramming of aged cells into iPSCs is possible, it has also been reported to be considerably more difficult than that from young donors. Our next step is to induce iMSCs from these aged mouse-derived iPSCs and investigate their differences compared with primary MSCs from aged mice as well as MSCs from young mice. In this study, we report the method used for cell sorting and evaluate its efficiency.

FIGURES:

Figure1.
Experimental Methods

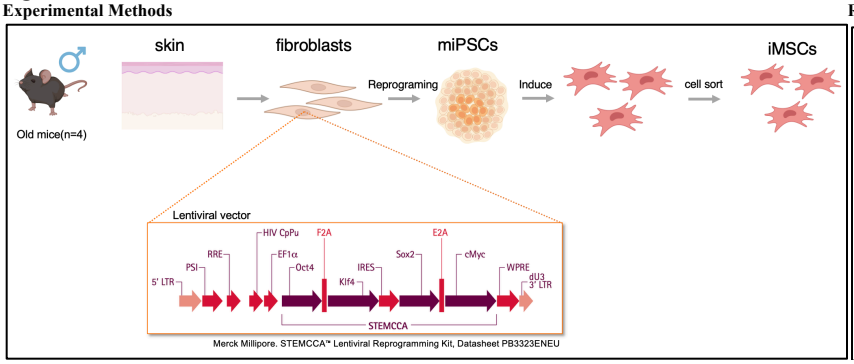

Figure2
Reprogramming Efficiency Of iPSCs from Aged Skin Fibroblats

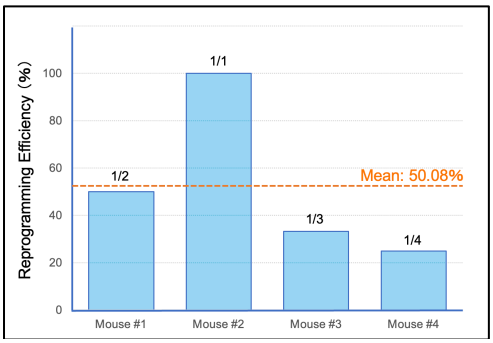

Figure3.
(a) Colony morphology of the established iPSC lines **(b) Immunocytochemistry (ICC) staining of iPSC cell line (#2)**