

## Evaluation of osteogenic and bone regenerative potential of mesenchymal cells derived from the gingival tissue of older adult

Pedro C. Aravena<sup>1,2</sup> Francisco Rivera<sup>2</sup>, Gerardo Méndez<sup>1</sup>, María Elena Silva<sup>2</sup>, Sergio Uribe<sup>1,3</sup>

<sup>1</sup> *School of Dentistry. Faculty of Medicine. Universidad Austral de Chile. Valdivia, Chile.*

<sup>2</sup> *Interdisciplinary Center for Nervous System Studies (CISNe). Faculty of Medicine. Universidad Austral de Chile. Valdivia, Chile.*

<sup>3</sup> *Bioinformatics Research Unit & BBCE, Riga Stradins University, Riga, Latvia.*

**INTRODUCTION:** Obtaining autologous stem cells is costly and fraught with post-surgical complications. Local and global reports have found Gingival-derived Mesenchymal Stem Cells (GMSCs) to be a reliable source with desirable properties.<sup>1</sup> Our purpose is to evaluate if GMSCs isolated from older patients in vitro maintain osteogenic potential and display typical MSCs properties.

**METHODS:** We performed an analysis of GMSCs phenotypes with CD markers analysis (CD105, CD90, CD73) and cell viability of gingival samples from the voluntary subjects: five older patients (mean  $70.3 \pm 3.2$  years; 3 women), five young patients (mean 20 years, two women) and five umbilical cord Mesenchymal Stem Cell samples as the positive control group. The viability of GMSCs from older vs. younger patients by MTT assay was determined. Samples GMSCs were cultured and MTT activity was measured at different times (24; 48; 72; 120, and 168 hours). We tested the osteogenic differentiation potential GMSCs of older patients. GMSCs were incubated in osteogenic for 21 days and, after that, Alizarin Red S staining was performed. Finally, we measured cell proliferation with immunohistochemistry technique.

**RESULTS:** Statistically similar positive markers for MSCs and viability of GMSCs were observed (Anova  $p < 0.05$ ; Fig. 1). The osteogenic potential of older GMSCs with the formation of calcium deposits shown in phase contrast image and with Alizarin Red S staining was verified (Fig. 2).

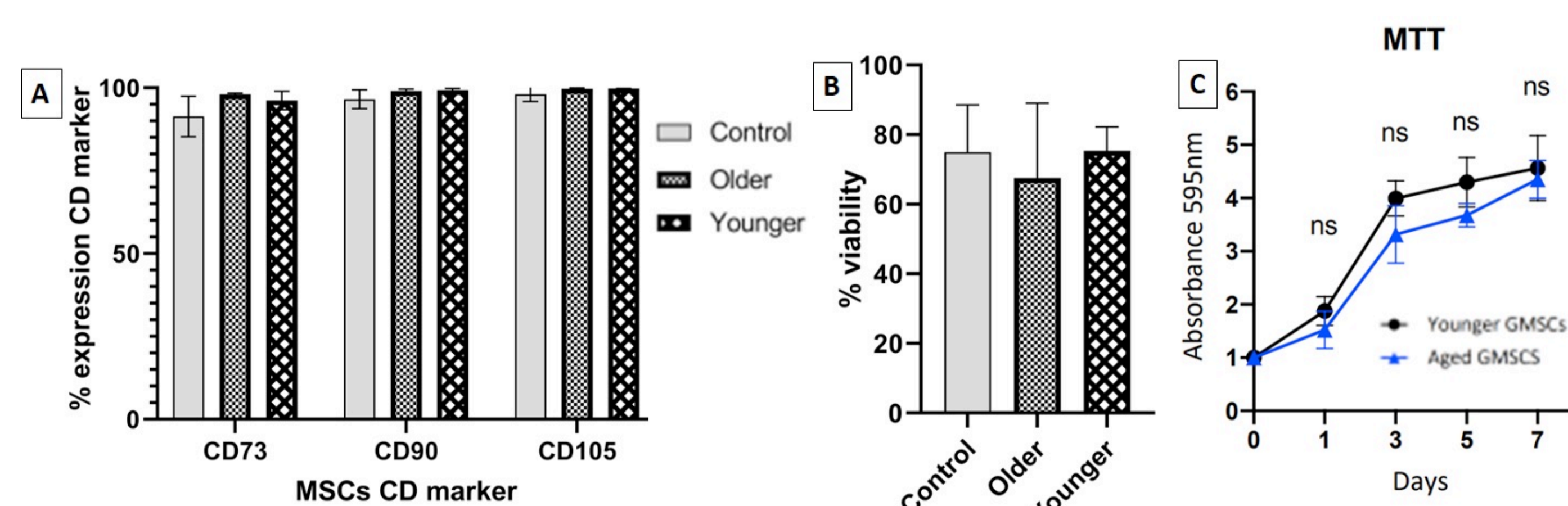


Fig. 1: image of CD stem cell marker tests (A), cell viability samples (B) over culture time (C)

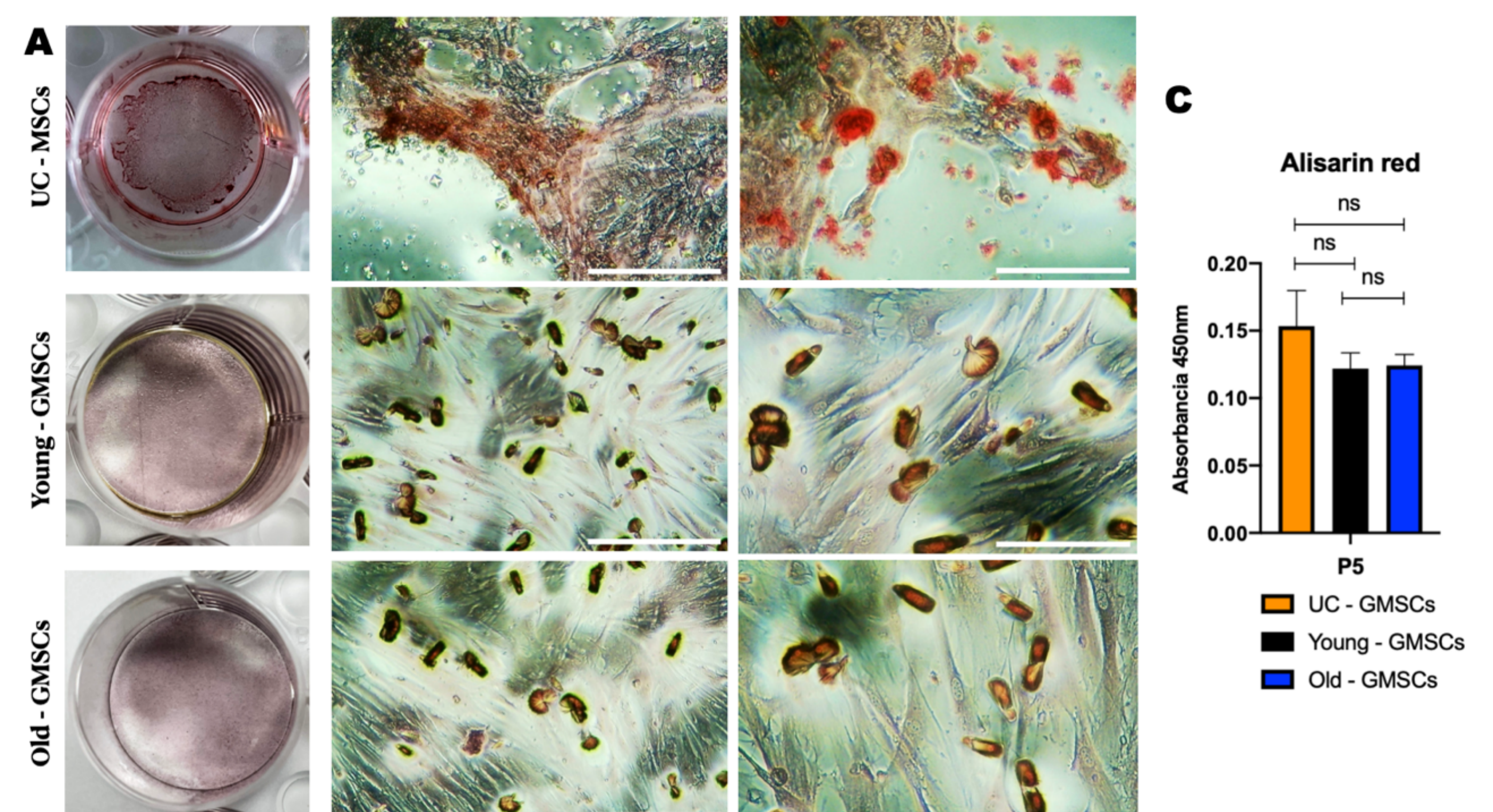


Fig 2: The calcium deposits formation in culture medium with Alizarin Red S staining (X20) and their level absorbance (C) were similar between study groups (Anova  $p < 0.05$ ).

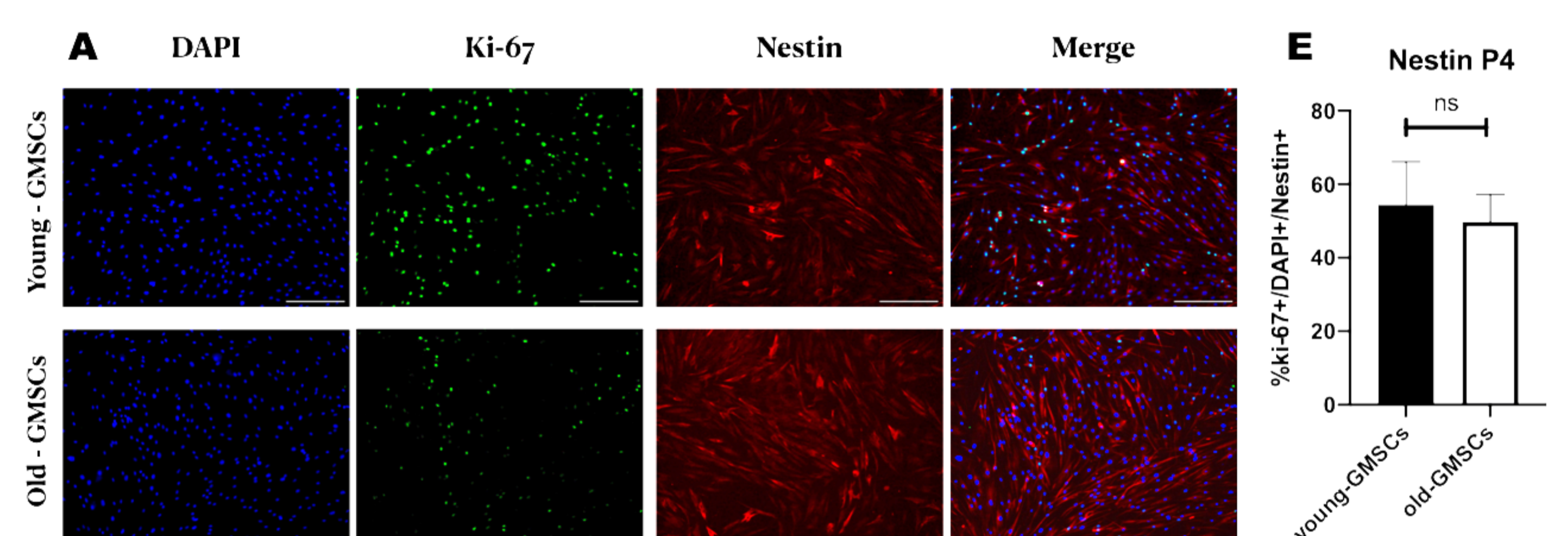


Fig 3: Cell nucleus proliferation measurement markers (Ki-67+ DAPI+ NESTIN+). The results showed similar rate proliferation between older and younger GMSCs study group (Anova  $p < 0.05$ ).

**DISCUSSION & CONCLUSIONS:** Expanded GMSCs display a typical MSCs marker expression profile, and GMSCs obtained from older patients retain cell viability, proliferative capability, and osteogenic potential. GMSCs from older subjects could be a cellular autologous therapy for bone tissue formation.

**REFERENCES:** <sup>1</sup>Tomasello, L., et al. (2017). Mesenchymal stem cells derived from inflamed dental pulpal and gingival tissue: a potential application for bone formation. Stem Cell Res Ther.1;8(1):179.