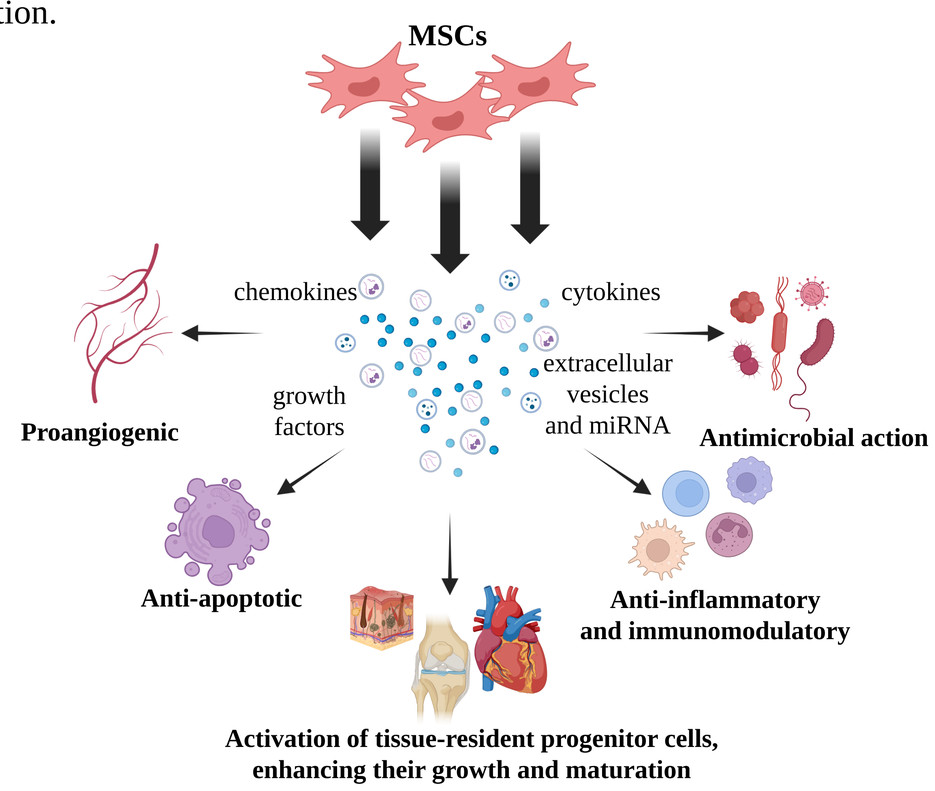
Conditioned medium (CM) from human adipose tissue derived mesenchymal stem/stromal cells (HATMSC2) protects vascular smooth muscle cells from calcification

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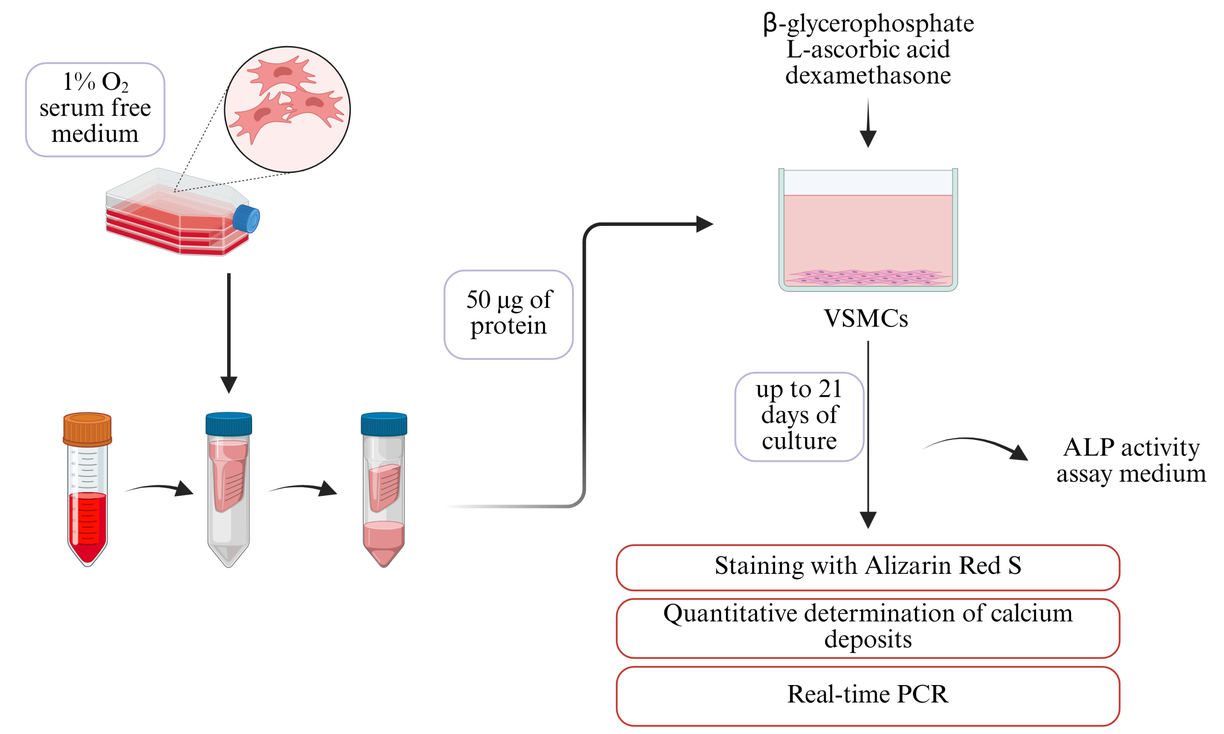
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Introduction

Heart valve calcification is a major global health concern, particularly among the elderly often requiring valve replacement. While mesenchymal stem/ stromal cells (MSCs) show promise for regeneration, their direct clinical use is limited by poor survival, regulatory requirements and high costs. Therefore, research is focusing on the MSC conditioned medium (CM), a complex mixture of bioactive factors that mediates the cells' therapeutic effects through paracrine mechanisms. The aim of this study is to determine anti-calcification effect of human adipose tissue-derived MSC (HATMSC2) CM in the *in vitro* model of vascular smooth muscle cells (VSMCs) calcification.



Methods



The HATMSC2 derived CM was produced by 24 hours incubation of cells in ischemic conditions, then concentrated and measured for total protein concentration. 50 µg of CM protein was added to VSMC cultures in calcifying medium containing 10mM β-glycerophospate and its effect was examined by the following: the amount of calcium deposits using Alizarin Red S staining, CPC; alkaline phosphatase (ALP) activity; relative quantification of early osteogenic markers: osterix (*OSX*), collagen type 1 (*COL-1*) and late osteogenic marker- osteocalcin (*OCN*); osteopontin (OPN) expression examined by immunofluorescent staining in VSMCs cultured in organic calcifying medium.

Results

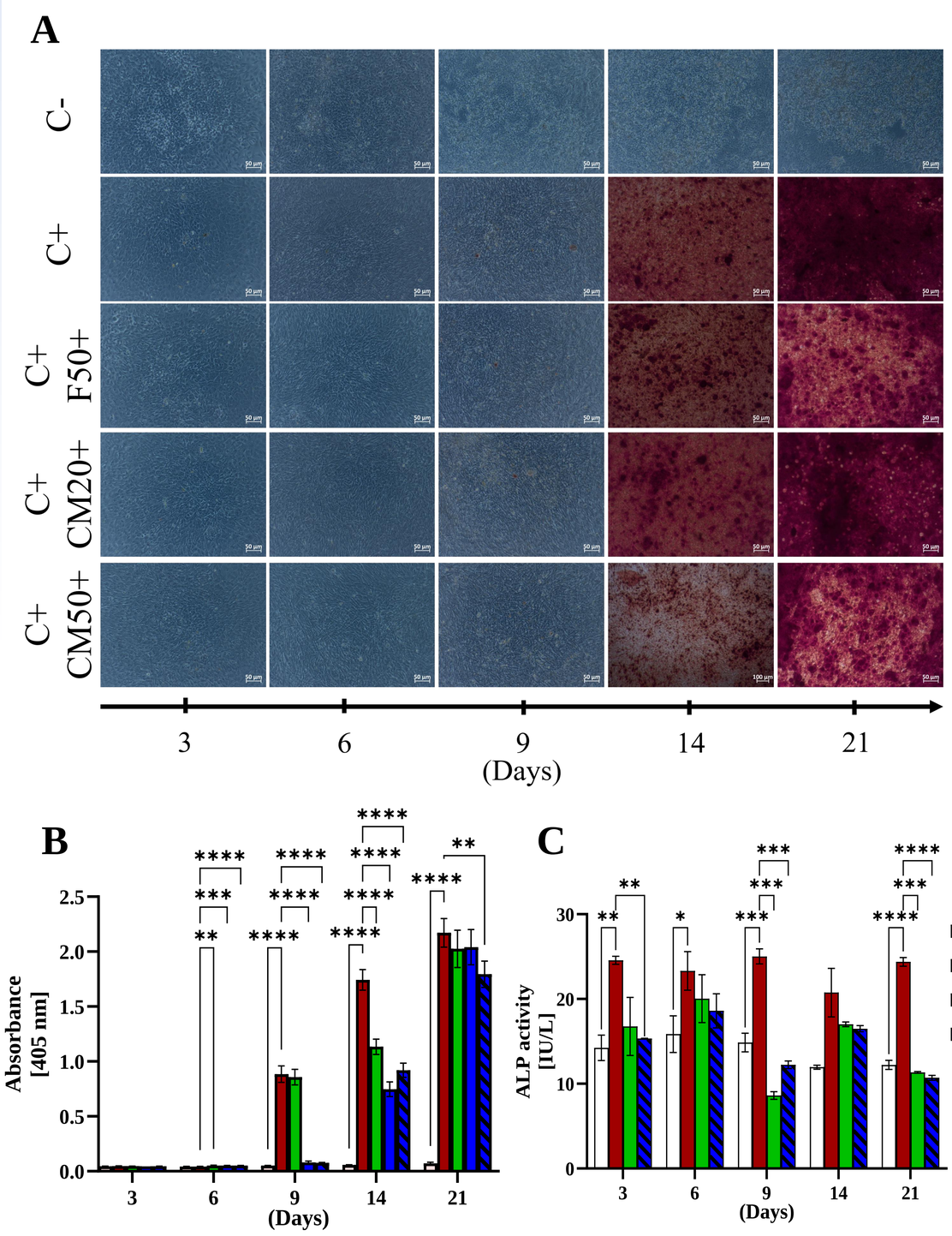


Figure 1. Visualisation of calcium deposits in VSMCs cultured in organic calcifying medium. Representative (**A**) microscopy images of Alizarin Red S saining (red). (**B**) Quantification of staining and (**C**) ALP activity was measured in three separate experiments; each performed in triplicate. Cells cultured in the standard culture medium were used as negative control (C-), cells cultured in organic calcifying medium were used as positive control (C+), cells cultured in organic calcifying medium with: 50 µg/mL of fetuin (C+F50+), 20 µg/mL of HATMSC2-CM (C+CM20+) and 50 µg/mL of HATMSC2-CM (C+CM50+). Data represent mean ±SEM, n = 3, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Statistical significance was compared to C+.

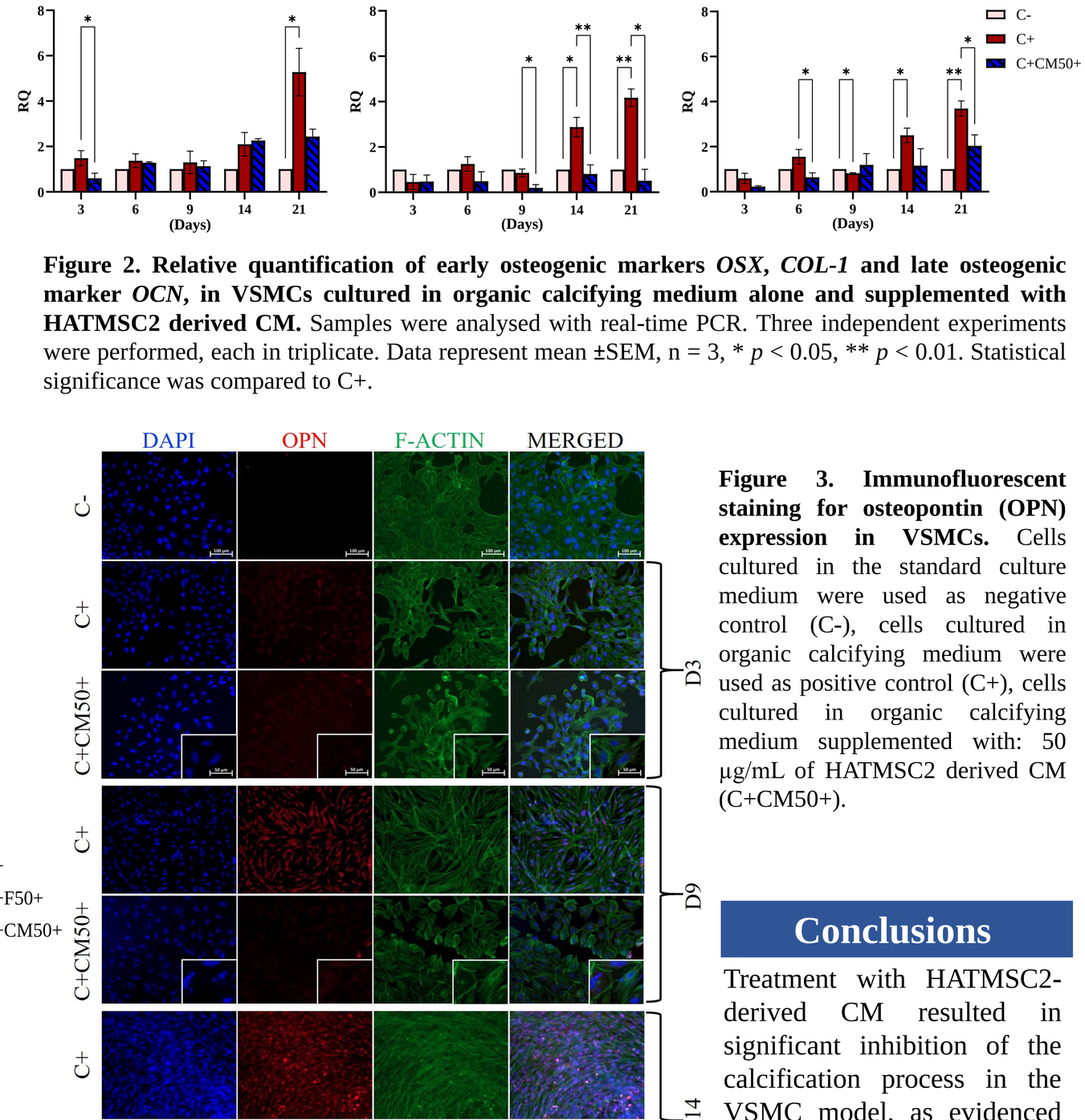


Figure 2. Relative quantification of early osteogenic markers *OSX*, *COL-1* and late osteogenic marker *OCN*, in VSMCs cultured in organic calcifying medium alone and supplemented with HATMSC2 derived CM. Samples were analysed with real-time PCR. Three independent experiments were performed, each in triplicate. Data represent mean ±SEM, n = 3, * $p < 0.05$, ** $p < 0.01$. Statistical significance was compared to C+.

Figure 3. Immunofluorescent staining for osteopontin (OPN) expression in VSMCs. Cells cultured in the standard culture medium were used as negative control (C-), cells cultured in organic calcifying medium were used as positive control (C+), cells cultured in organic calcifying medium supplemented with: 50 µg/mL of HATMSC2 derived CM (C+CM50+).

Conclusions

Treatment with HATMSC2-derived CM resulted in significant inhibition of the calcification process in the VSMC model, as evidenced by our results. This indicates that the use of HATMSC2-CM as an anti-calcifying therapeutic agent is a promising approach to protect heart valve from harmful conditions.

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