Effect of vitamin C and A in the differentiation of Mesenchymal Stem Cells derived from dental pulp.

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INTRODUCTION

Recently, some strategies for the acceleration of dental movement have been studied, which allow to shorten the times of orthodontic treatment, reducing the secondary effects that can occur such as caries, periodontal disease and root resorption among others. Within these mechanisms of acceleration is found the use of different chemical substances such as vitamins, which can alter bone remodeling, inducing the differentiation of osteoblasts and / or osteoclasts.

Some previous studies have suggested an important role of vitamins A (vit A) and C (vit C) in the stimulation of osteoblastic differentiation and extracellular matrix synthesis.

However, the evidence is controversial, so more studies are needed to determine the effect of these vitamins on bone remodeling and tooth movement.

OBJETIVE

To determine the effect of vit A and C on the proliferation and differentiation of mesenchymal cells obtained from dental pulp (hDPSC) .

MATERIALS AND METHODS

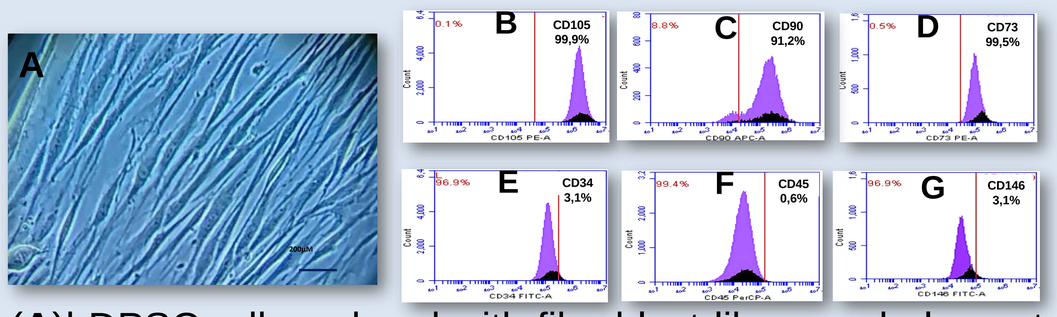
Isolation and characterization of hDPSC

Cell culture and Vitamin A, Vitamin C and Vitamin A+C treatment

- **Changes in cell number and viability** → **Resazurin metabolic assay and trypan blue exclusion test.**
- **Morphological changes** → **Light microscopy**
- **Cell differentiation**
  - **Calcification nodules formation** → **Alizarin red staining quantification assay**
  - **Alkaline phosphatase activity**
  - **Expression of RUNX2** → **RT-qPCR**
- **Statistic analysis** → ***T test*, ANOVA and Tukey test.**

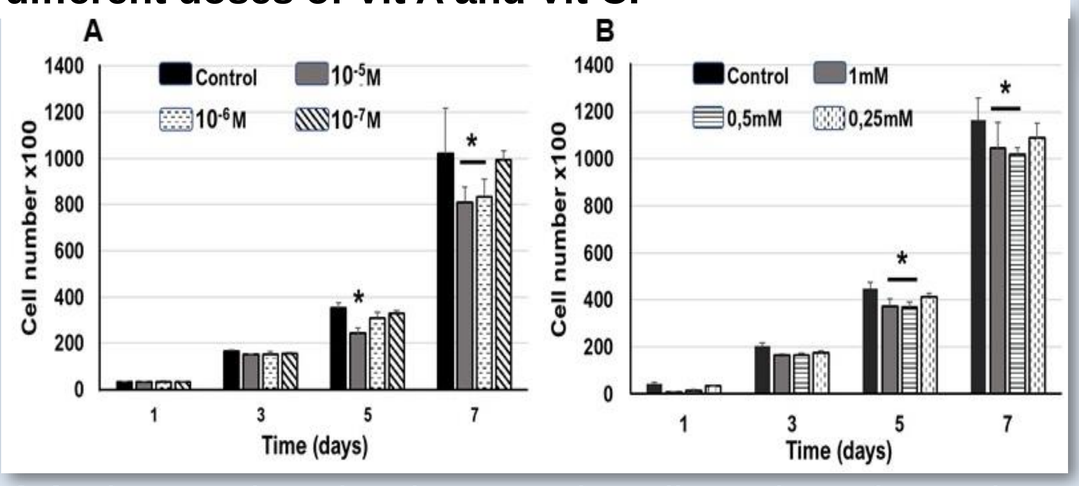
RESULTS

Isolation and characterization of hDPSC:



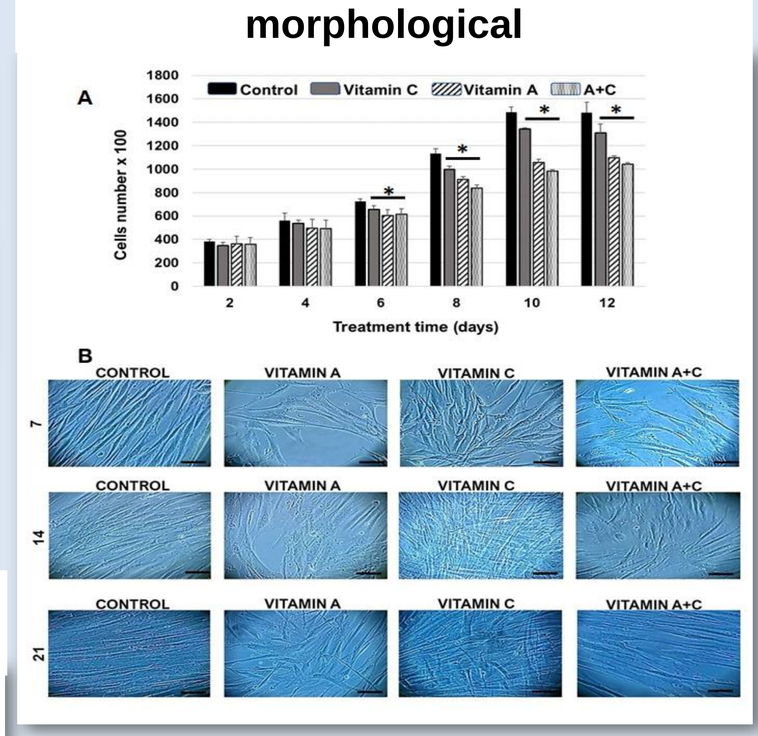
(**A**)hDPSC adhered and with fibroblast-like morphology at 10 days of culture. Flow cytometry histograms with positive surface markers for CD90 (**B**), CD105 (**C**), CD73 (**D**) and negative for CD34 (**E**), CD45 (**F**) and CD 146 (**G**).

Reduction in the number of hDPSC by treatment with different doses of Vit A and Vit C.



(A) Vit A produced a decrease in cell number after 5 days of treatment with 10^{-5} M and at 7 days with 10^{-5} M and 10^{-6} M. **(B)** Vit C reduced the number of hDPSC treated with 1mM and 0.5mM doses after 5 and 7 days of treatment. Asterisks show significant differences (p<0.05).

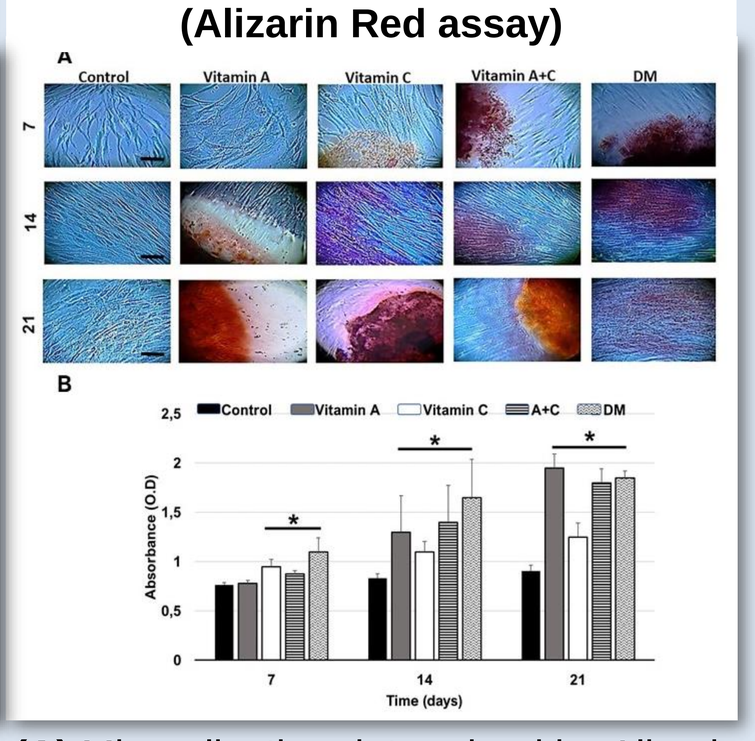
Changes in cell number and morphological



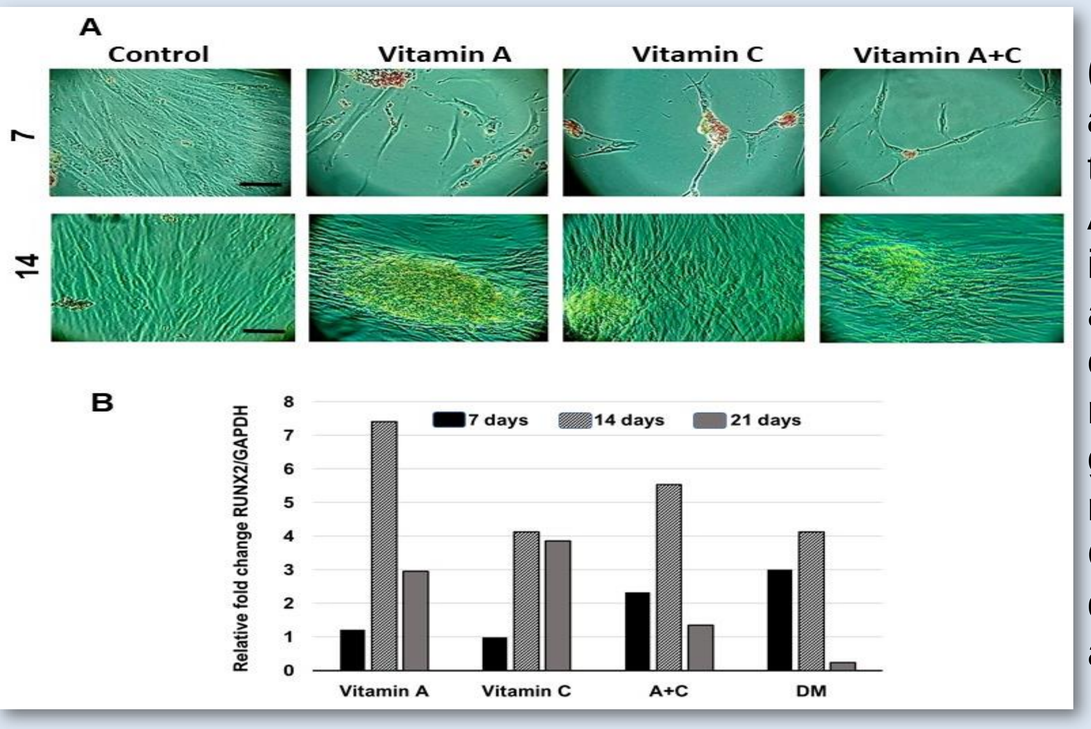
(A) Treatment with Vit C (0.5 mM), Vit A (10^{-5}M) and the two vitamins simultaneously (A+C) produced a significant reduction in the number of cells from day 6 of treatment relative to the control group. * p<0.05.

(B) Photomicrograph of hDPSC cultures treated with vit A, vit C and A+C for 7, 14 and 21 days. Bar: 200μm.

Extracellular matrix mineralization (Alizarin Red assay)



(A) Mineralization determined by Alizarin red S staining. Strong matrix staining was observed in the photomicrographs, which indicated the apparent formation of calcification nodules. Bar: 200μm. **(B)** Measurement of absorbance of Alizarin red S stain extracted from cells under different treatments at 7, 14 and 21 days. Differentiation medium (DM). *P <0.05.



(A) Staining of hDPSC cells with alkaline phosphatase: Cells without treatment (Control), and treated with vit A, vit C and A+C, were immunohistochemically stained with alkaline phosphatase at 7 and 14 days of treatment. **(B) Quantification of the relative expression of the RUNX2 gene:** The data are expressed in relation to the expression levels of the GAPDH gene and cells treated with differentiation medium (DM) were analyzed as a positive control.

CONCLUSIONS

Treatment with vit A, C and A+C induced a significant reduction in proliferation, produced morphological changes, and caused an increase in RUNX2 expression, indicating stimulation of differentiation of hDPSCs towards osteoblastic lineage.

Vit C had a lesser effect on proliferation and gene expression; however, it produced morphological changes and calcification nodules early.

The effect that prevails when combining the two vitamins is generated by vit A alone.