The Dentin Scaffold can induce osteogenic differentiation of CGF cells

Laura Giannotti[1], B. Di Chiara Stanca[2], F. Damiano[1], E. Stanca[1], A. Palermo[1], C. Demitri[1] and L. Siculella[1]

1. Laboratory of Molecular Biology, Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy;
2. Department of Institute of Polymers, Composites and Biomaterials, National Research Council (IPCB-CNR), 80125 Naples, Italy

Introduction

CGF (Concentrated Growth Factors) is a fibrin-based biomaterial enriched with growth factors. It is obtained by centrifuging venous blood at different speeds using the Silfradent device [1].
Regarded as a novel autologous biological matrix, it has been demonstrated to contribute to bone regeneration in vivo, as well as promote osteoblast differentiation [2, 3]. Integrating scaffold materials with stem cell-based strategies is a crucial approach in tissue regeneration. In this context, the osteogenic differentiation of CGF-derived primary cells has been investigated using CGF combined with hydroxyapatite–silicon scaffolds [4].
In the present study, we employed dentin scaffolds to further assess the osteogenic potential of CGF-derived primary cells.

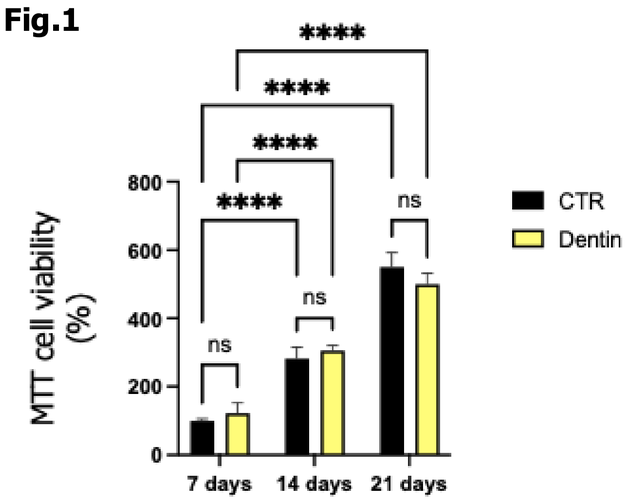
Materials and Methods

CGF was prepared from 8 mL of the blood sample. Briefly, each blood sample was centrifuged, and three fractions were obtained. CGF (the middle dense fraction) was the one used for the experiments. After three days, CGF was chopped into small pieces to improve the release of primary cells; these pieces were plated on Dentin scaffolds and cultured with L-DMEM (Basal medium, BM) or Osteogenic Medium (OM, L-DMEM with 10 mM β-glycerophosphate, BGP, and 100 µM ascorbic acid 2-phosphate, AA) for 21 days. The cellular viability of primary cells cultivated on Dentin scaffolds was determined by MTT assay. Moreover, the matrix mineralization of CGF primary cells cultivated on Dentin scaffolds was evaluated through alizarin red staining and mRNA quantification of osteogenic differentiation markers by real-time PCR.

Results

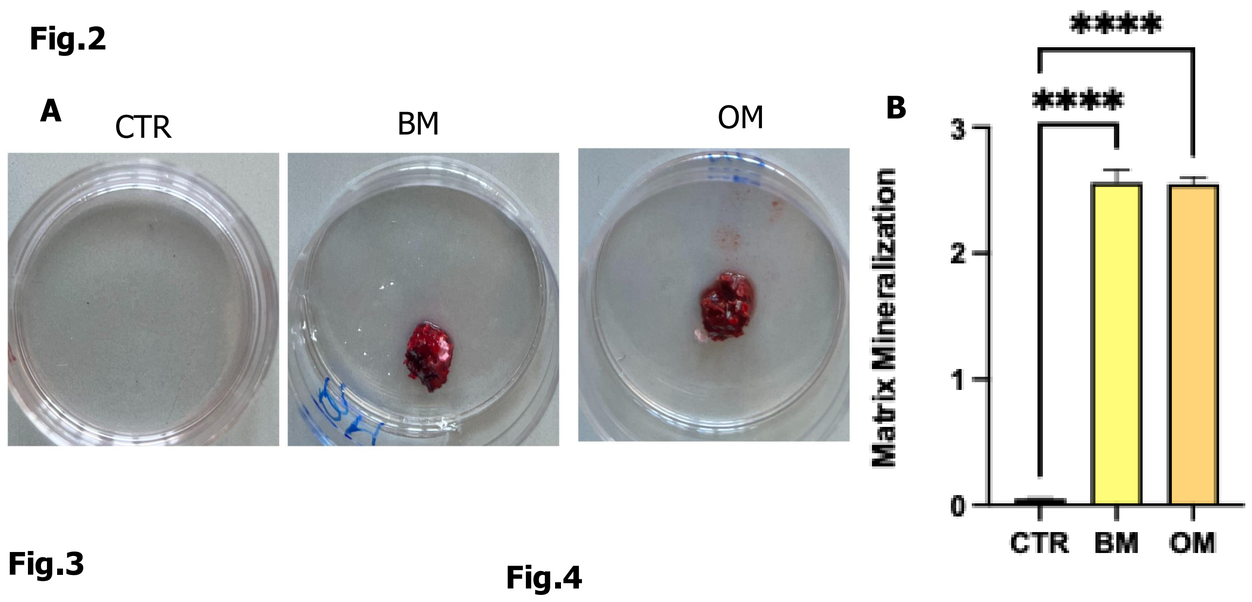
Figure 1 shows the results of the MTT assay comparing hBMSCs cultured under standard two-dimensional conditions (CTR) with hBMSCs seeded on dentin scaffolds (Dentin). The data clearly indicate that cells grown on dentin exhibit a progressive increase in cell viability/metabolic activity at different time points, with values higher than the control condition, although not statistically significant. This suggests that the dentin scaffold supports and promotes cell proliferation or at least maintains the cells in a metabolically active state over time.

Fig.1



In order to measure the ability of the Dentin scaffolds to induce the osteogenic differentiation of hBMSCs, the Alizarin red assay (Fig.2A) was set up. As reported, CTR in hBMSc in 2D culture, BM is the hBMSCs seeded on the scaffold for 21 days with the basal medium and OM condition, where we use an osteogenic medium with the addition of substrates as B-glycerophosphate and Ascorbic Acid, which causes the formation of a mineralized matrix in hBMSCs in vitro after 21 days of culture. Percentage of matrix mineralization of the three conditions normalized with DNA concentration. hBMSCs grown on Dentin scaffolds in BM show a significant increase in density values compared to the control (Fig.2B).
These data suggest that in the presence of the Dentin scaffolds, hBMSc can form a mineralized structure even in the absence of the inducers of the osteogenic process.

Fig.2



We examined the molecular level the osteogenic differentiation of hBMSCs induced by the Dentin scaffold. The expression of three osteogenic markers was quantified: RUNX2 and Osteocalcin (OCN) and COL1A1 IN BM and OM conditions (Fig.3). At the same time, the expression of characteristic stem cell markers was also quantified: CD105 and CD45 (Fig.4).

As regards the analysis of stem cell markers, the results reported show that when compared to control cells, the expression of genes for CD105 and CD45 is significantly lower in cells grown on the Dentin scaffold in both conditions. And the expression levels of typical osteogenic differentiation genes are significantly higher in the cells incubated with the scaffolds compared to the control cells.

Fig.3

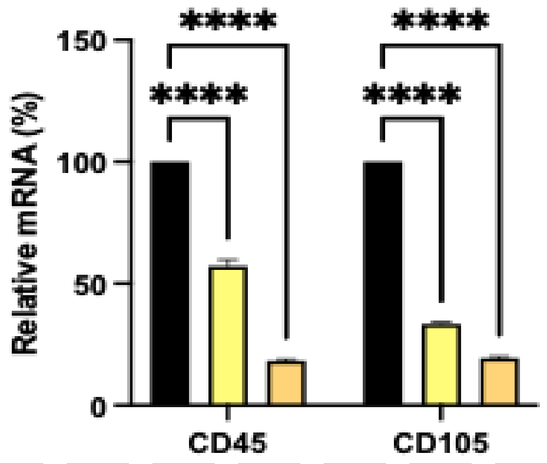
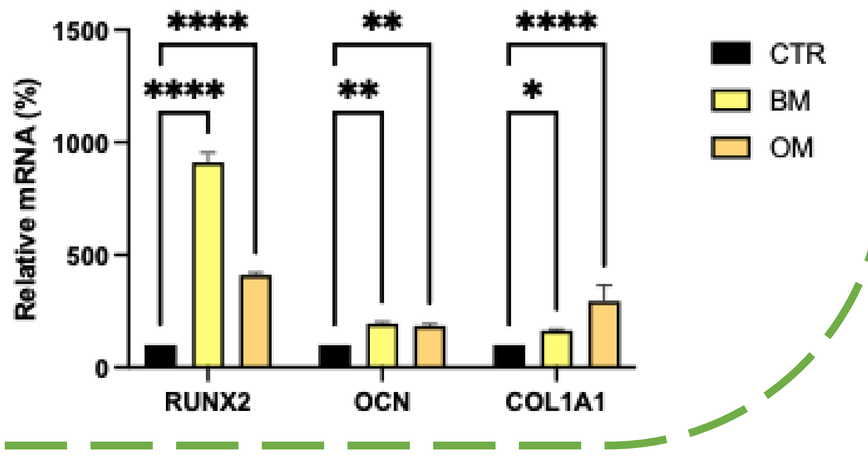


Fig.4



Conclusions

The osteogenic effects of CGF fractions have been extensively studied. CGF was cultured in the presence of Dentin scaffolds, and our findings demonstrate that Dentin scaffolds are non-cytotoxic and support the adhesion, growth, and proliferation of CGF-derived cells. Importantly, these scaffolds promote osteogenic differentiation, highlighting their potential as effective biomaterial supports for CGF-based regenerative applications.

REFERENCES
1. Bernardi S, Mummolo S, Tecco S, Continenza M.A, Marzo G. Histological characterization of Sacco's concentrated growth factors membrane. Int. J. Morphol. **2017**, *35*, 114.
2.Stanca E, Calabriso N, Giannotti L, Nitti P, Damiano F, Stanca BDC, Carluccio MA, De Benedetto GE, Demitri C, Palermo A, Ferrante F, Siculella L, Rochira A. Analysis of CGF Biomolecules, Structure and Cell Population: Characterization of the Stemness Features of CGF Cells and Osteogenic Potential. Int J Mol Sci. **2021**, 22, 8867.
3. Rochira A, Siculella L, Damiano F, Palermo A, Ferrante F, Carluccio M.A, Calabriso N, Giannotti L, Stanca E. Concentrated Growth Factors (CGF) Induce Osteogenic Differentiation in Human Bone Marrow Stem Cells. Biology **2020**, *9*, 370.
4. Giannotti, L.; Di Chiara Stanca, B.; Nitti, P.; Spedicato, F.; Damiano, F.; Demitri, C.; Calabriso, N.; Carluccio, M.A.; Palermo, A.; Ferrante, F.; et al. Hydroxyapatite–Silicon Scaffold Promotes Osteogenic Differentiation of CGF Primary Cells. *Biology* **2023**, *12*, 528.