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Osteogenic differentiation of CGF cells can be induced by Silicon scaffold

Laura Giannotti[1], B. Di Chiara Stanca[1], P. Nitti[2,3], F. Damiano[1], C. Demitri[2], F. Spedicato[1], A. Palermo[4], F. Ferrante[5], N. Calabriso[6], M.A. Carluccio[6], L. Siculella[1]

1. Laboratory of Molecular Biology, Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy;
2. Department of Engineering for Innovation, University of Salento, Lecce, Italy;
3. Analytical and Isotopic Mass Spectrometry Laboratory, Department of Cultural Heritage, University of Salento, Lecce, Italy;
4. Associate Professor in Implant Dentistry College of Medicine and Dentistry, Birmingham B4 6BN, UK;
5. Specialist in Oral Surgery, Private Practioner, Lecce, Italy;
6. National Research Council (CNR) Institute of Clinical Physiology (IFC), Lecce, Italy.

Introduction

A new autologous biological matrix involved in bone regeneration in vivo [1] and osteoblast differentiation [2] is Concentrated Growth Factor (CGF). CGF is a fibrin biomaterial rich in growth factors obtained by centrifugation of venous blood, at alternating speeds, as set on the Silfradent device [3]. The application of scaffolding materials together with stem cell technologies plays a key role in tissue regeneration. Therefore, in this study we used CGF with scaffolds made of hydroxyapatite and silicon (HA-Si). The aim was to evaluate osteogenic differentiation of CGF primary cells induced by HA-Si scaffolds.

Materials and Methods

For the preparation of the CGF, 8 ml of blood sample was taken. Briefly, each blood sample was centrifuged and three fractions are 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 HA-Silicon 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 HA-Si scaffolds was determined by MTT assay and a structural characterization of this complex was performed by SEM analysis. Moreover, the matrix mineralization of CGF primary cells cultivated on HA-Si scaffolds was evaluated through alizarin red staining and mRNA quantification of osteogenic differentiation markers by real-time PCR.

Results

1. Preliminary Cell Viability Evaluation

As shown in Fig.1, the number of viable cells increases up to 21 culture days, confirming that the HA-Si scaffold is not toxic and allows the proliferation of the cells that adhere to it.

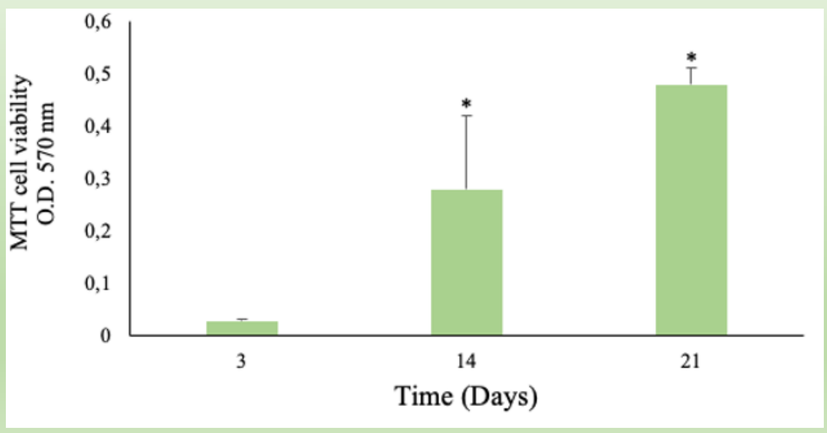

Fig.1

2. Effect of CGF and HA-Si scaffold on Matrix Mineralization

Fig.2 shows that the scaffold itself is capable of inducing osteogenic differentiation in BMSCs (CTR). In the presence of the HA-Si scaffolds, CGF primary cells grown in BM show a significant increase in optical density values compared to the control.

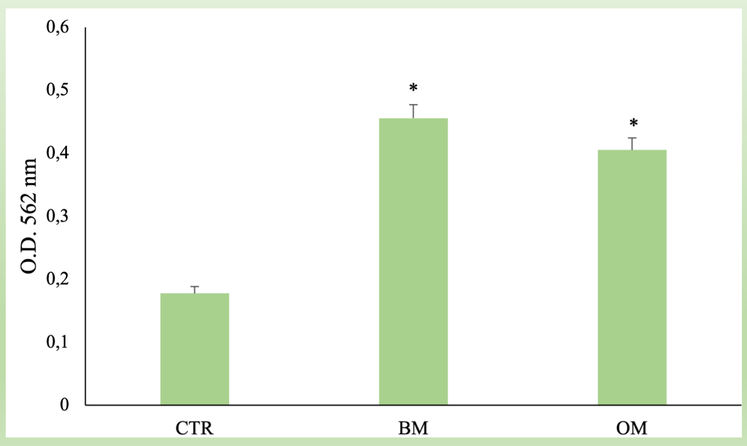

Fig.2

3. Effects of CGF on surface markers expression and osteogenic differentiation markers

Fig.3 shows that the expression levels of CD105, CD90 and CD73 are significantly lower in cells incubated with the HA-Si scaffold compared to control. At the same time, Fig.4 shows the expression levels of RUNX2 and OCN significantly higher in cells incubated with HA-Si scaffold.

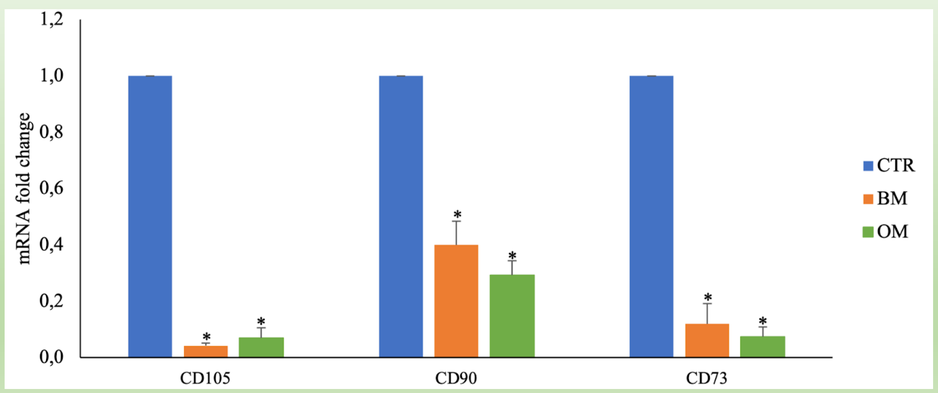

Fig.3

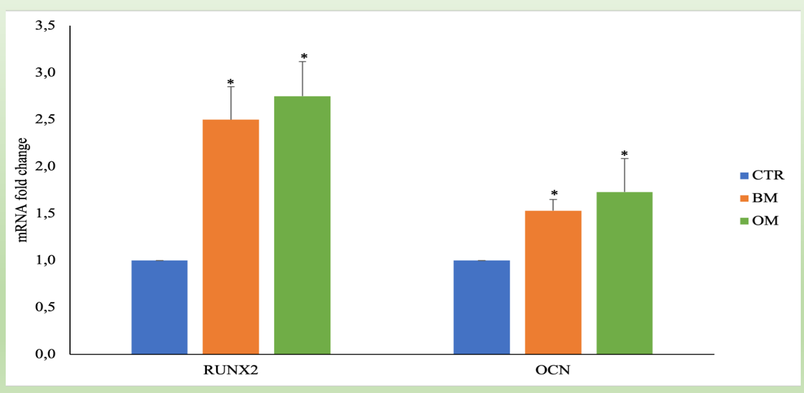

Fig.4

4. Structural characterization of CGF primary cells grown on HA-Si scaffolds

As reported in Fig.5b, cells released by CGF are able to adhere and colonize the HA-Si scaffold. With respect to Fig.5a, the formation of a cross-linked structure, probably of a protein nature, is observed in Fig.5b. A mineralized structure, is observed in Fig.5c, due to the addition of the inducers of osteogenic differentiation, that allows the formation of calcium and phosphate crystals, typical of this process.

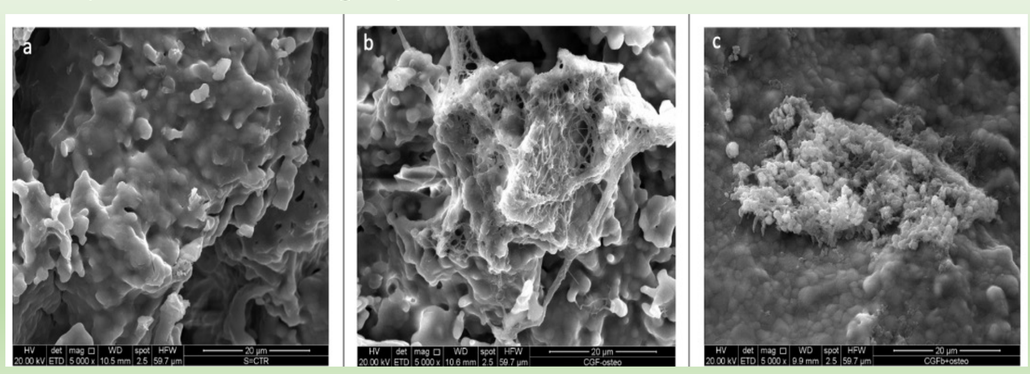

Fig.5

Conclusions

The osteogenic effects of CGF fractions have been extensively studied. CGF was cultured in presence of HA-Si scaffolds and then we analyzed:

- The cells viability of CGF primary cells, proving that the HA-Si scaffold is non-toxic and allows the proliferation of adhering cells.
- The effect of CGF and HA-Si scaffold on matrix mineralization, proving that CGF primary cells grown in BM show a significant increase in optical density values compared to the control. Therefore, the scaffold is sufficient to provide the complete mineralization of primary cells without OM.
- The effects of CGF on surface markers and osteogenic differentiation markers: the expression of CD105, CD90 and CD73 genes is significantly lower in cells incubated with the HA-Si scaffold, instead RUNX2 and OCN mRNA levels are significantly higher, suggesting an osteogenic differentiation of CGF primary cells.
- The structural characterization of CGF primary cells grown on HA-Si scaffolds allowed to observe the formation of a cross-linked structure in CGF and HA-Si scaffold cultivated in BM.

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