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Chemical, structural and biological characterization of CGF and analysis of its osteogenic potential

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INTRODUCTION

In the field of regenerative medicine, there is growing interest in platelet concentrates derived from whole blood to improve tissue regeneration processes [1]. Concentrated growth factors (CGF) is the latest generation of platelet derivatives and it's produced by centrifugation of the blood sample at alternating speed rates [2]. This process leads to a dense fibrin matrix, which contains more growth factors than the other platelet derivatives and it has been used to induce osteogenic differentiation in hBMSC[3]. The aim of this work was the chemical, structural and biological characterization of CGF.

RESULTS

1. Molecular characterization

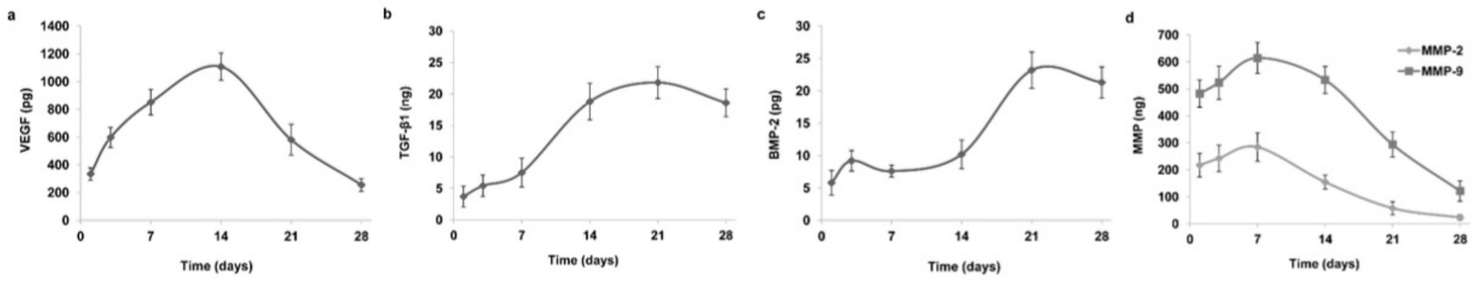
GC/MS metabolomics analysis highlighted the high concentration of L-glutamic acid and taurine in CGF compared to Platelet Poor Plasma (PPP) (Tab. 1).

Tab. 1

Compound	R.T. (min)	Qion (m/z)	Concentration (mg/L)	
			CGF	PPP
L-Glutamic	17.640	432	0.56 * ± 0.04	0.06 ± 0.00
Taurine	14.154	296	3.82 * ± 0.11	0.08 ± 0.02

Growth factors release was assessed by ELISA. They were gradually released over time up to 28 days from CGF: VEGF was released slowly up to 14 days and gradually decreased (Fig. 1a); TGF-β1 (Fig. 1b) and BMP-2 (Fig. 1c) were also released slowly, they peaked at 21 days and their values remained high up to 28 days; MMP-9 and MMP-2 (Fig. 1d) were released faster and peaked after 7 days, then gradually decreased.

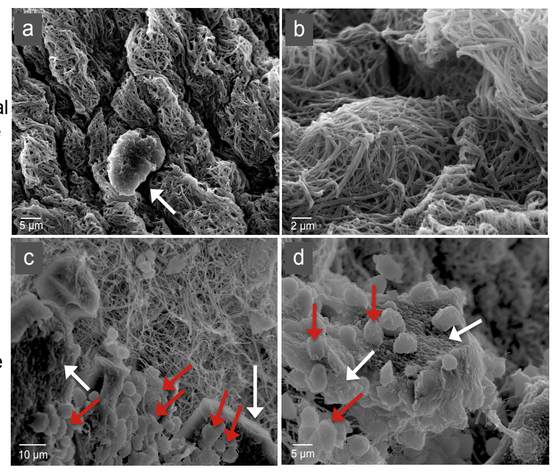
Fig. 1



2. Structural characterization

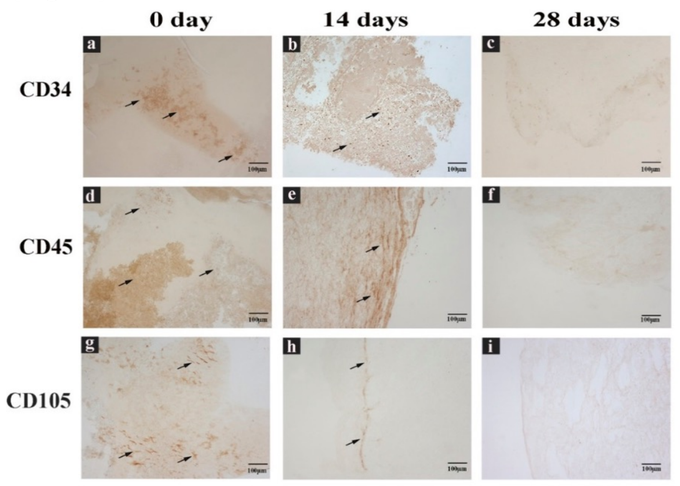
SEM observation (Fig. 2) did not reveal the presence of cells on the surface of CGF but showed a fibrin framework denser than inside of CGF, where large populations of cells were present.

Fig. 2



Immunohistochemistry analysis of CGF showed a very uniform distribution of nucleated cells entrapped in the fibrin network. The sections reacted positively to CD34, CD45, and CD105 immunolabelling (Fig. 3).

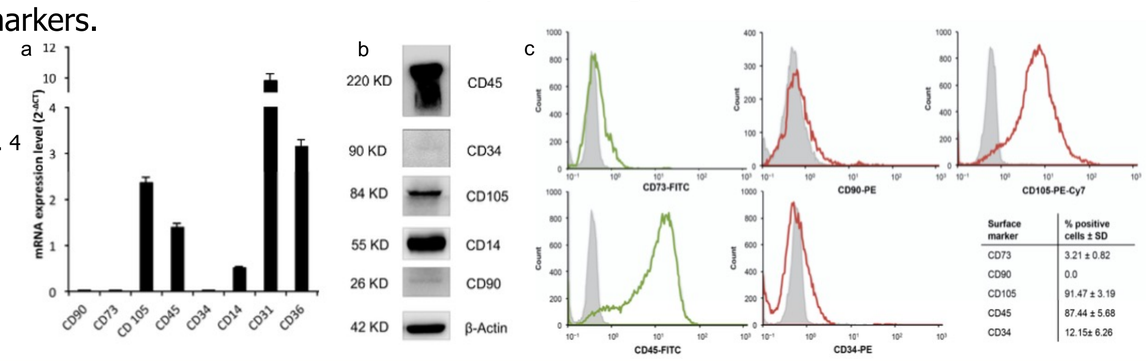
Fig. 3



3. Biological characterization

CGF primary cells were isolated for the identification of surface markers by real-time PCR (Fig. 4a), Western blot (Fig. 4b) and flow cytometry (Fig. 4c).
Adherent cells released from CGF expressed high level of CD105 and CD45 surface markers.

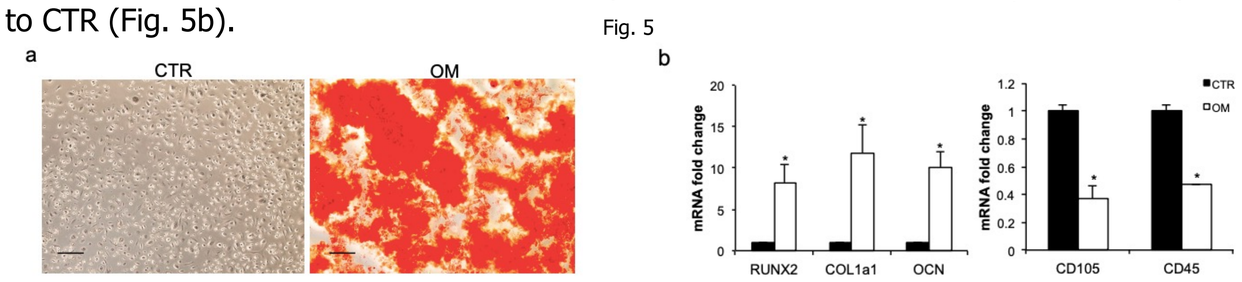
Fig. 4



4. Osteogenic differentiation

The osteogenic differentiation of CGF primary cells was evaluated after 21 days of culture in osteogenic medium (OM) through matrix mineralization by Alizarin red staining (ARS) and mRNA quantification of specific markers by real-time PCR.
CGF primary cells showed a very strong ARS (Fig. 5a), an increase in RUNX2, COL1a1 and OCN mRNA levels and a decrease in the expression of CD105 and CD45, when compared to CTR (Fig. 5b).

Fig. 5



CONCLUSIONS

CGF represents a new autologous blood-derived biomaterial, attracting growing interest in the field of regenerative medicine. In this study, the chemical, structural and biological characterization of CGF was achieved to deepen the knowledge of this very promising biomaterial.

- GC/MS metabolomics analysis highlighted the high concentration of L-glutamic acid and taurine in CGF. They both have been shown to have positive effects on osteogenic differentiation and influence bone metabolism.
- Growth factors and MMPs were gradually released over time up to 28 days from CGF preparation, following specific release kinetics. The present findings reported a continuous and prolonged release of multiple bioactive factors over time, suggesting that CGF is suitable in promoting the complex and long process of tissue regeneration.
- By SEM and immunohistochemistry, we analyzed the fibrin structure of CGF and we started a first characterization of the cells present on the inside. These cells seemed to migrate from the center, where fibrin network was less dense to the peripherical area of the sections, where fibrin appeared to be more densely intertwined. This could explain why the release of cells from CGF seemed to be rather slow.
- Adherent cells released from CGF expressed high level of CD105 and CD45 surface markers, suggesting mesenchymal cell features and so the ability to differentiate into different cell lines.
- To better characterize the use of CGF in the field of regenerative medicine, the ability of these cells to differentiate into osteoblasts was tested. We found that CGF primary cells were able to differentiate into osteoblasts, as demonstrated by the formation of mineralized nodules, the expression of the osteogenic markers and the loss of stem cell markers.

These data, taken together, highlight interesting new perspectives for the use of CGF in tissue regeneration and in regenerative medicine.

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