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Human Periapical Cysts-Mesenchymal Stem Cells Cultured with Allogenic Human Serum are a "clinical-grade" construct alternative to bovine fetal serum and indicated in the regeneration of endo-periodontal tissues

Le human periapical cysts-mesenchymal stem cells coltivate con siero umano allogenico costituiscono un costrutto ''clinical-grade'' alternativo al siero fetale bovino ed indicato nella rigenerazione dei tessuti endo-parodontali

Marco Tatullo [a,*], Massimo Marrelli [a], Francesca Palmieri [a], Carlo Rengo [b], Francesco Paduano [a], Gianrico Spagnuolo [b]

[a] *Tecnologica Research Institute, Stem Cell Unit, Crotone, Italy*
[b] *Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Naples, Italy*

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Regenerative medicine;
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Translational research.

Abstract
Aim: Our research investigated the use of human serum (HS) as a safe and clinical-grade culture medium, using a new cell-model: hPCy-MSCs. This article is aimed to concretely applicate the concept of "waste-based regenerative dentistry" to translate it in future endo-periodontal applications.
Methodology: HPCy-MSCs were cultured in 2 different mediums, both containing α-MEM: the 1st with 10% FBS (Control group), and the 2nd with 10% human serum (Test group).

* Corresponding author at: Tecnologica Research Institute, Street Enrico Fermi, 88900 Crotone, Italy. Tel./fax: +39 0962930414.
E-mail: marco.tatullo@tecnologicasrl.com (M. Tatullo).
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Cell proliferation and stemness assays, gene expression, immunophenotypic analysis and osteogenic differentiation were performed to verify our hypothesis. cDNA samples were amplified with qPCR.

Experiments were performed in triplicate and analysed with statistical software.

Results: The hPCy-MSCs cultivated in a medium with HS were morphologically similar to those cultivated with FBS, and showed a significantly higher proliferation rate. Von Kossa's staining revealed that osteoblasts from hPCy-MSCs in HS implemented with osteogenic induction factors, showed a better osteogenic activity, also confirmed by a significant upregulation of osteopotin (OPN) and matrix extracellular phosphoglycoprotein (MEPE).

Conclusions: HPCy-MSCs cultivated in HS showed phenotypic stability and a clear regenerative binding, thus, suggesting these two components as a clinically-grade construct for future endo-periodontal therapies.

PAROLE CHIAVE
Medicina rigenerativa;
Cellule staminali;
Osteogenesi;
Human periapical cyst-MSCs;
Ricerca traslazionale.

Riassunto

Obiettivi: La nostra ricerca ha analizzato l'utilizzo del siero umano (HS) come mezzo di coltura sicuro e ''clinical-grade'', per uso clinico, utilizzando un nuovo modello cellulare: le hPC-MSCs. Questo articolo ha lo scopo di applicare concretamente il concetto di ''odontoiatria rigenerativa basata sui rifiuti biologici'', al fine di tradurlo in future applicazioni endo-periodontali.

Materiali e metodi: Le HPCy-MSCs sono state coltivate in 2 mezzi di coltura diversi, entrambi contenenti α-MEM: il primo con 10% di FBS (gruppo di controllo) e il secondo con il 10% di siero umano (gruppo di test).

Sono stati eseguiti saggi di proliferazione cellulare e di staminalità, espressione genica, analisi immunofenotipica e differenziamento osteogenico per verificare la nostra ipotesi di partenza. Campioni di cDNA sono stati amplificati con qPCR.

Gli esperimenti sono stati eseguiti in triplicato e analizzati con software statistici.

Risultati: Le hPC-MSC coltivate in un terreno con HS erano morfologicamente simili a quelle coltivate con FBS e mostravano un tasso di proliferazione significativamente più alto. La colorazione di Von Kossa ha rivelato che gli osteoblasti da hPC-MSC coltivate in HS implementato con fattori di induzione osteogenica hanno mostrato una migliore attività osteogenica, confermata anche da una significativa up-regolazione di osteopotina (OPN) e fosfoglicoproteina della matrice extracellulare (MEPE).

Conclusioni: Le HPCy-MSC coltivate in HS hanno mostrato stabilità fenotipica e un chiaro atteggiamento rigenerativo, suggerendo quindi questo protocollo come un approccio clinicamente valido per le future terapie endo-periodontali.

Introduction

The discovery of human periapical cysts-mesenchymal stem cells (hPCy-MSCs) has, for the first time, introduced dentistry in the concept of ''regenerative waste medicine'', the regenerative medicine obtainable from the reuse of biological waste to exploit it the clinical potential. HPCy-MSCs showed excellent ability to differentiate between osteogenic phenotypes and surprisingly toward the nervous tissue.[1–3]

Stem cell regeneration is, however, limited in clinical practice for the use of fetal bovine serum (FBS), used as a nutritional supplement in culture media. In fact, its xenogenic origin does not make FBS secure in patients applications. Despite several MSCs from different sources,[4] and a number of scaffold[5–12] have been already described and investigated in the literature, the main limitation to clinical applications seems to be the standardization of clinical-grade procedures, to overcome immunological and biological concernings.

In the light of the studies in the literature,[13–16] our research analyzed for the first time human serum (HS) as a safe and ''clinical-grade'' alternative in regenerative medicine, using a new cellular model: hPCy-MSCs, opening innovative scenarios on the concept of ''waste regenerative dentistry'' in future endo-periodontal applications.

Materials and methods

HPCy-MSCs were obtained as described in literature[1,3] and cultured in a medium containing α-MEM with 10% FBS (Gibco) in the Control group, and with 10% human serum (HS, Sigma) in the test group. The HS used in this study (AB plasma) is used as by the supplier protocol.

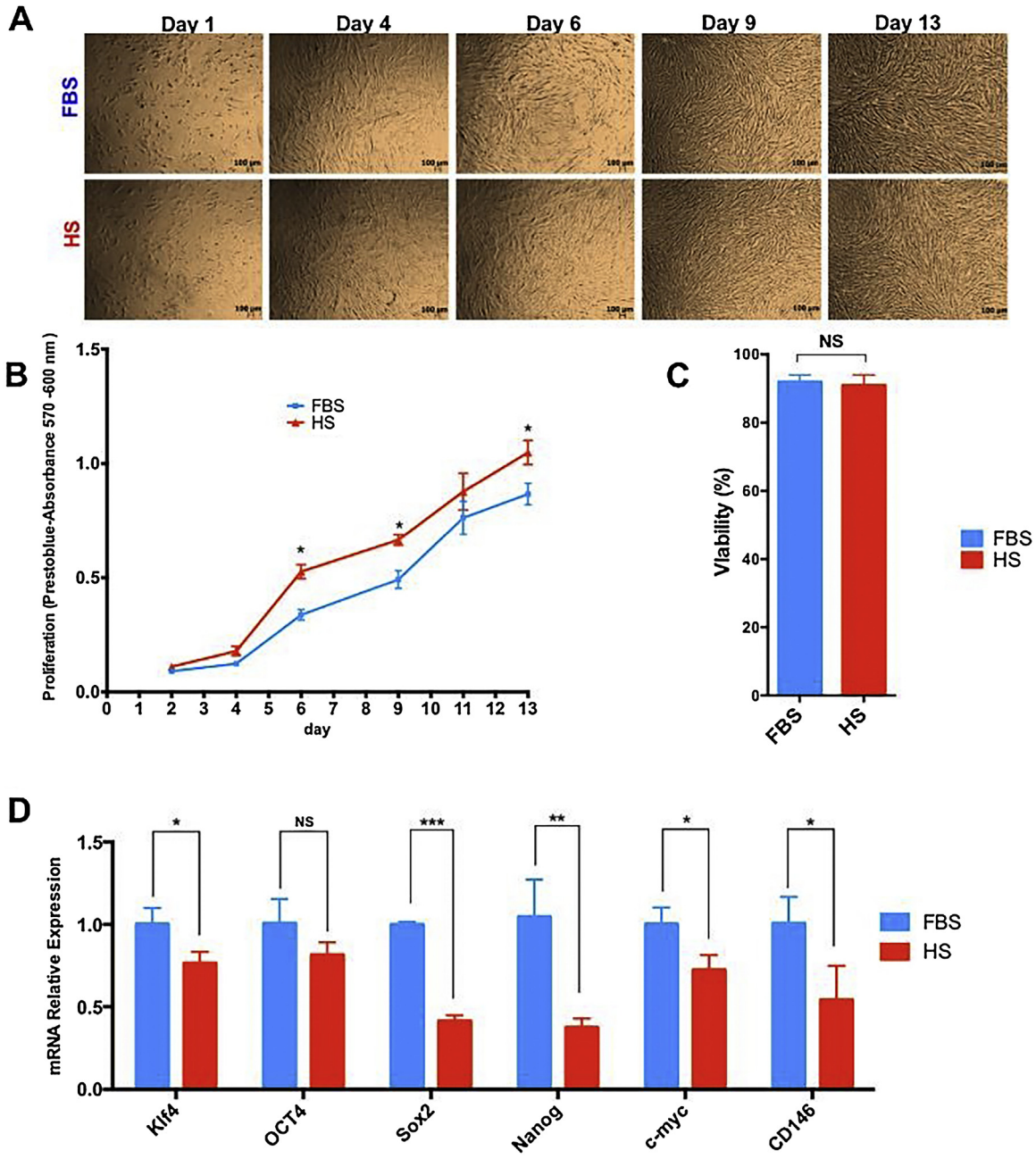

Figure 1 Proliferation, vitality, and expression of stem molecular markers in hPCy-MSCs grown in HS or FBS. (A) Characteristic morphology of hPCy-MSCs cultivated in 10% HS or 10% FBS (days 1—13). (B) Cell growth assay of hPCy-MSCs cultured in 10% HS or 10% FBS. $^{*}P < 0.05$. (C) Cell viability of hPCy-MSCs cultured in 10% HS or 10% FBS. (D) Expression of mRNA for the genetic indications of staminality: Klf4, OCT4, Sox-2, Nanog, c-myc and the CD146 pericitary marker in hPCy-MSCs cultured in 10% HS or 10% FBS analyzed by qRT-PCR. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, N.S., not significant. FBS, fetal bovine serum; HS, human serum.

Cell proliferation tests, stem cell assays, gene expression, immunophenotypic analysis and osteogenic differentiation tests were performed with quantitative analysis to verify our hypothesis. CDNA samples were amplified with qPCR using sequences of primers.

Experiments were performed in triplicate, and data evaluated with GraphPad Prism software (statistically significant differences with: $^{*}P < 0.05$; $^{**}P < 0.01$; $P < 0.001$; N.S. = not significant).

Results

The hPCy-MSCs cultivated in a medium with HS were morphologically similar to those cultivated with FBS (Fig. 1A); however, hPCy-MSCs showed a significantly higher proliferation rate, from day 6 (Fig. 1B). Under both culturing conditions, the amount of vital hPCy-MSCs was about the same (>95%, Fig. 1C). The expression of the main stemness genes has been verified (Fig. 1D).

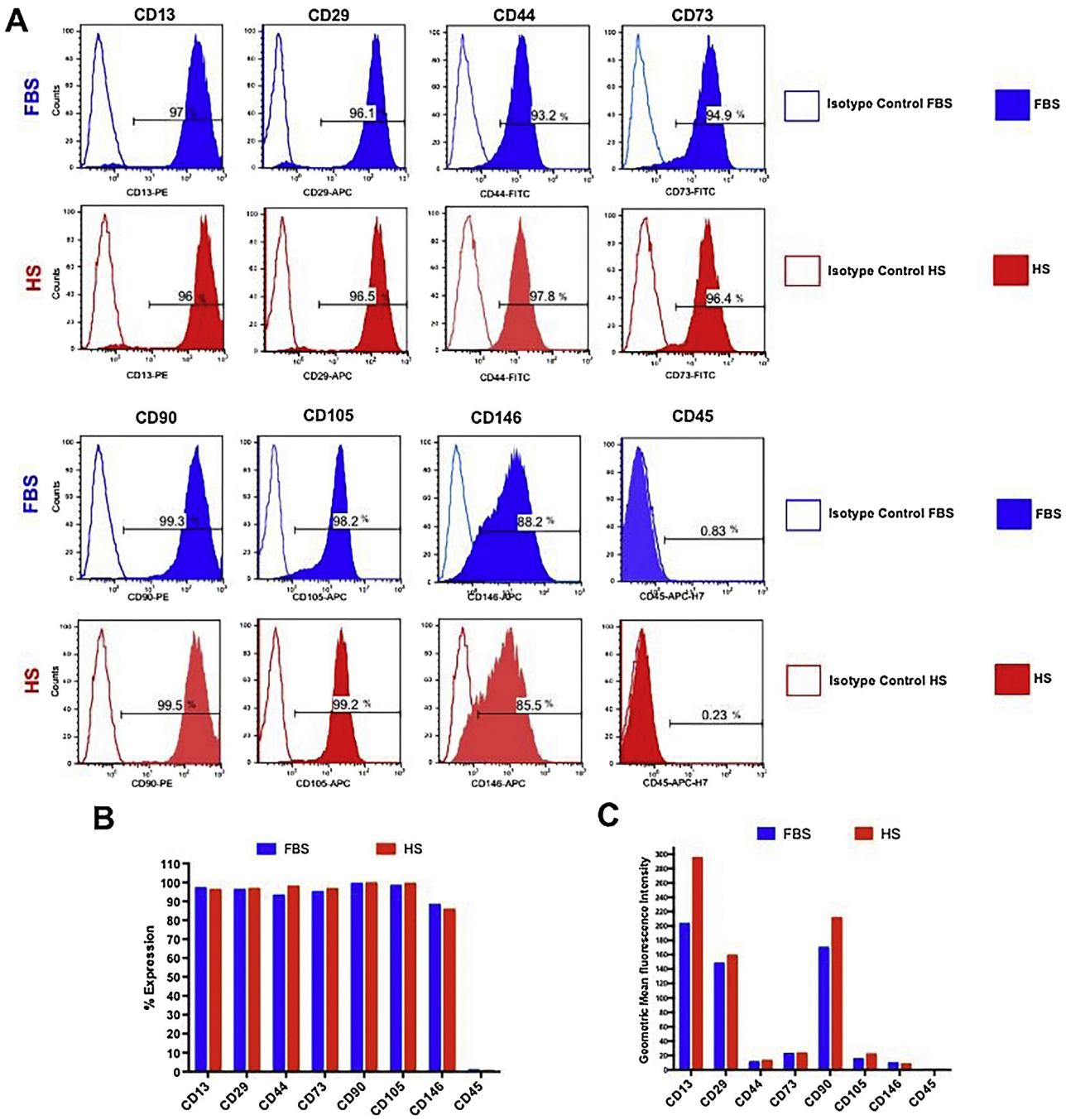

Figure 2 Expression of surface stem markers in hPCy-MSCs cultivated in HS or FBS. (A) Expression of surface stem markers: CD13, CD29, CD44, CD73, CD90, CD105, CD146 and CD45 in hPCy-MSCs cultivated in 10% HS compared with those grown in 10% FBS. The control of the isotype is indicated by the empty histogram, whereas the coloring of the antibodies is indicated by a solid histogram. (B) Percentage data of surface stem markers in hPCy-MSCs cultivated in 10% HS compared with those grown in 10% FBS. (C) Geometric mean fluorescence intensity of surface stem markers in hPCy-MSCs cultivated in 10% HS compared with those grown in 10% FBS. FBS, fetal bovine serum; HS, human serum.

Flow cytometry of phenotypic markers of hPCy-MSCs cultivated in HS and FBS (Fig. 2A) did not show statistically significant differences (Fig. 2B).

Von Kossa's staining revealed that hPCy-MSCs induced towards the osteogenic phenotype in HS, showed a greater differentiation than those cultivated in FBS (Fig. 3A and B). These observations were confirmed by a significant upregulation of osteopotin (OPN) and matrix extracellular phosphoglycoprotein (MEPE) in hPCy-MSCs cultivated in osteogenic and basal media containing HS (Fig. 3C).

Discussion

Patients treated with FBS-grown cells are exposed to a concrete risk of immunogenicity and the risk of transmission

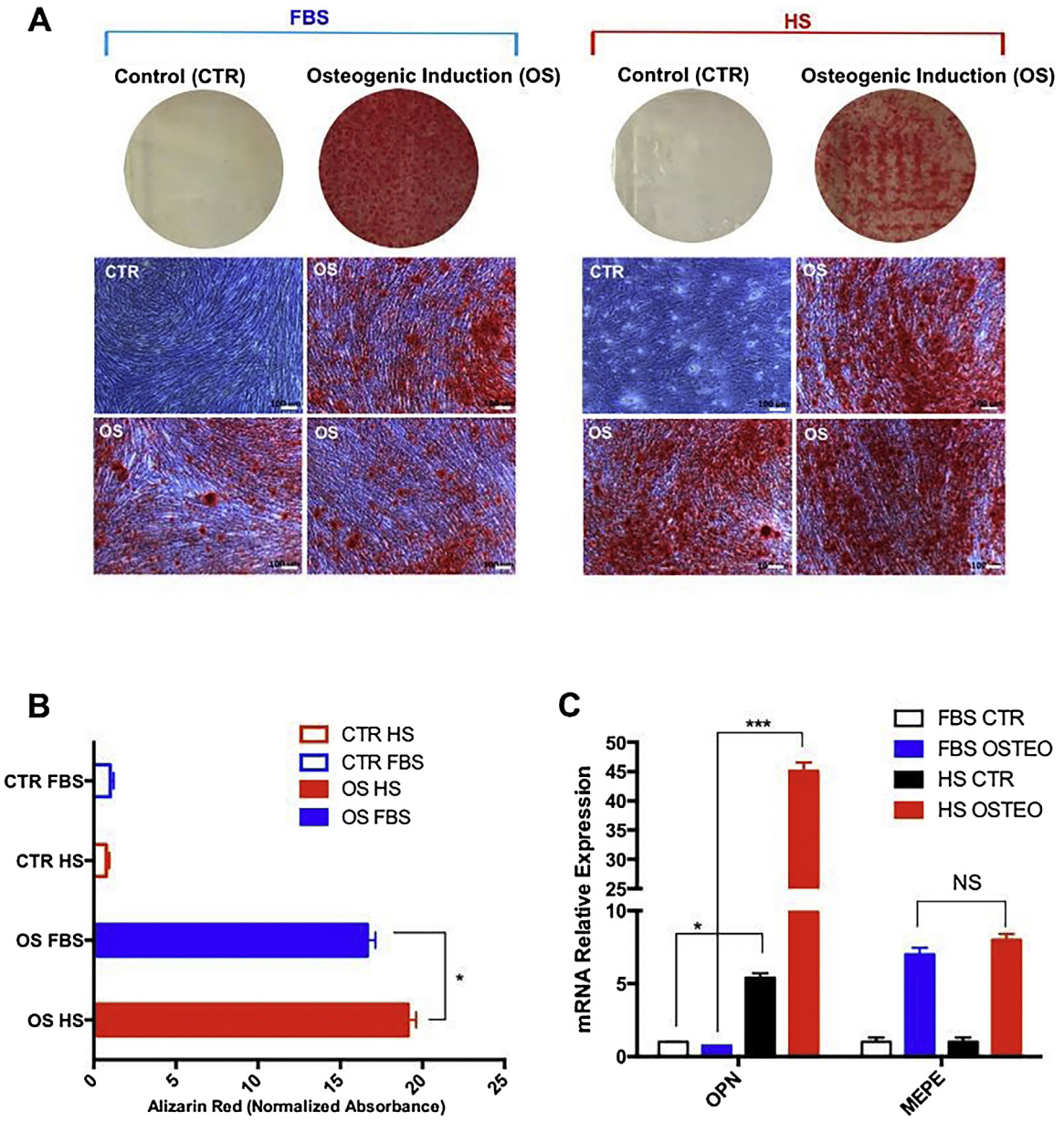

Figure 3 Osteogenic differentiation potential of hPCy-MSCs cultivated in HS or FBS. (A) Osteogenic differentiation of hPCy-MSCs, grown in HS, and compared with those grown in FBS, colored with red Alizarin. (B) Quantification by red blood alizarine test for the production of mineralized matrix in hPCy-MSCs cultivated in HS compared with those grown in FBS. *$P < 0.05$. (C) Expression of osteogenic markers mRNA: OSP and MEPE in hPCy-MSCs cultivated in HS compared to those cultured in FBS after 21 days of differentiation (OS). FBS, fetal bovine serum; HS, human serum; CTR, control; OS, osteogenic differentiation. *$P < 0.05$, ***$P < 0.001$, N.S., not significant.

of prion, bacterial or viral pathologies, making FBS dangerous for advanced cell therapy.[13]

For more than 30 years the scientific community has requested the use of alternative supplements to FBS[14] and in 2011 the European Commission, with the technical note EMA/410/01, suggested that animal medicines be avoided.

Recently, some authors have studied HS as an alternative to FBS on stem cell models of dental origin that are alternative to our[15,16]; however, the plasticity of hPCy-MSCs makes them ideal for regeneration to bone-like and neuron-like tissues.

Conclusions

HPCy-MSCs cultivated in HS showed excellent skills towards bone regeneration, also avoiding the risks derived from the use of FBS, thus, suggesting these two components as a clinically-grade construct for future endo-periodontal therapies.

Clinical relevance

Human periapical cysts-mesenchymal stem cells cultured with allogenic human serum are clinically usable, without

to use alternative xenogeneic serum. This ''clinical-grade'' procedure, together with the great plasticity of hPCy-MSCs and the recent experimental results reporting their ability to differentiate towards both bone-like, tooth-like and neuron-like tissues, makes the construct made from such a new MSCs and the ''safe'' culture medium an implantable biological device greatly indicated in the regeneration of endo-periodontal tissues.

Conflict of interest

We disclose no conflict of interest.

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