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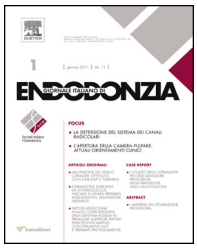

ORIGINAL ARTICLE/ARTICOLO ORIGINALE

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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

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KEYWORDS
Regenerative medicine;
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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).

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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.

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