Cytotherapy 2013 Aug;15(8):920-9 Epub 2013 Apr 24

PMID: 23623274 DOI: 10.1016/j.jcyt.2013.01.219

Production of human platelet lysate by use of ultrasound for ex vivo expansion of human bone marrow-derived mesenchymal stromal cells

Martina Bernardi, Elena Albiero, Alberta Alghisi, Katia Chieregato, Chiara Lievore, Domenico Madeo, Francesco Rodeghiero, Giuseppe Astori

Abstract

Background aims: A medium supplemented with fetal bovine serum (FBS) is of common use for the expansion of human mesenchymal stromal cells (MSCs). However, its use is discouraged by regulatory authorities because of the risk of zoonoses and immune reactions. Human platelet lysate (PL) obtained by freezing/thawing disruption of platelets has been proposed as a possible substitute of FBS. The process is time-consuming and not well standardized. A new method for obtaining PL that is based on the use of ultrasound is proposed.

Methods: Platelet sonication was performed by submerging platelet-containing plastic bags in an ultrasonic bath. To evaluate platelet lysis we measured platelet-derived growth factor-AB release. PL efficiency was tested by expanding bone marrow (BM)-MSCs, measuring population doubling time, differentiation capacity and immunogenic properties. Safety was evaluated by karyotyping expanded cells.

Results: After 30 minutes of sonication, 74% of platelet derived growth factor-AB was released. PL enhanced BM-MSC proliferation rate compared with FBS. The mean cumulative population doubling (cPD) of cells growth in PL at 10%, 7.5% and 5% was better compared with cPD obtained with 10% FBS. PD time (hours) of MSCs with PL obtained by sonication was shorter than for cPD with PL obtained by freezing/thawing (18.9 versus 17.4, $P < 0.01$). BM mononucleated cells expressed MSC markers and were able to differentiate into adipogenic, osteogenic and chondrogenic lineages. When BM-MSCs and T cells were co-cultured in close contact, immunosuppressive activity of BM-MSCs was maintained. Cell karyotype showed no genetic alterations.

Conclusions: The proposed method for the production of PL by sonication could be a safe, efficient and fast substitute of FBS, without the potential risks of FBS.