PLATELET LYSATE FOR THE EXPANSION OF HUMAN NUCLEUS PULPOSUS CELLS

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Purpose

Advanced Therapy Medical Products for intervertebral disc regeneration require a clinically meaningful cell number obtained by *ex vivo* expansion. Fetal Bovine Serum (FBS) has been used as the gold standard medium supplement, even if it is a potential xenogeneic source of antigenic agents and it could exhibit lot-to-lot variability. Recently, human blood derived products, such as Platelet Lysate (PL), are considered as a promising alternative, containing a pool of growth factors which promote cell growth. Aim of this work is to verify the PL efficiency during the expansion process of human nucleus pulposus cells; FBS was considered as a control.

Methods

Intervertebral disc samples (n = 3) were harvested from informed patients during microdiscectomy. The nucleus pulposus tissues were digested with collagenase overnight. The obtained NP cells were seeded onto flasks (10.000 cells/cm^2) with Dulbecco's Modified Eagle's Medium High Glucose, 10% FBS and antibiotics and, once reached sub-confluence, they were detached and divided into two aliquots: one cultured with medium containing 10% FBS and the other containing 5% PL (Lyset™ Kit) at the same cell density. Every 7 days, until passage 10, cultures were monitored under an optical microscope and Cellular Doublings (CD) were calculated. Results were analyzed using an ANOVA statistical model, considering CD as response variable, while type of supplement (PL or FBS), culture passage and cell line as fixed factors. Statistical significance was set at $p \leq 0.05$.

Results

A higher cell density was morphologically observed when NP cells were cultured in medium supplemented by PL. Cellular doubling was independent from the cell line, significantly higher in cultures with PL than FBS (1.68 *versus* 0.50; $p < 0.00001$) and significantly influenced by the passage ($p = 0.0070$): it was very low for the first passage (-0.21) and higher for the others ($\geq$ 0.59).

Conclusions

Human platelet lysate resulted an effective alternative to fetal bovine serum to support the *in vitro* NP cell expansion, avoiding xenoinfection and immunogenic risks. Further investigations will be focused on the use of PL for other cell lines (e.g. fibroblasts, mesenchymal stem/stromal cells) and in cryopreservation medium instead serum.

This work was supported by Cariplo_Regione Lombardia (StemDelivery ID 42617604); Lyset™ Kit was kindly supplied by Carlo Erba Reagents S.r.l.