

Lecture: good cell culture practice

## Alternatives to the use of fetal bovine serum: platelet lysates as serum replacement in cell and tissue culture

Caroline Rauch<sup>0</sup>, Elisabeth Feifel<sup>1</sup>, Harald Schöffl<sup>0</sup>, Walter Pfaller<sup>1</sup>, Gerhard Gstraunthaler<sup>1</sup>

<sup>0</sup> zet (Linz) (AT); <sup>1</sup> Innsbruck Medical University (Innsbruck) (AT)

e-mail: gerhard.gstraunthaler@i-med.ac.at

Fetal bovine serum (FBS) is commonly used as essential supplement to cell culture media. FBS is a cocktail of most of the factors required for cell attachment, growth, and proliferation *in vitro* and is thus used as an almost universal growth supplement effective with most types of human and animal cells. However, the use of animal serum also bears a number of disadvantages. These can either be seen from (1) a theoretical, cell biological point of view, since serum in general is an ill-defined, heterogeneous component in culture media, (2) from ethical perspectives in terms of animal protection arguments about harvest and collection of FBS from bovine fetuses, and (3) in terms of recent concerns about the global supply vs. demand of FBS. It is estimated that about 500,000 litres FBS are produced per year for the world market. This means, that more than 1,000,000 bovine fetuses have to be harvested, and it is expected, that these numbers will continue to increase annually. As a consequence, a number of strategies have been developed to reduce or replace the requirement for FBS in cell culture media. It is a well known fact, that natural clot serum rather than plasma promotes the growth and proliferation of cultured cells. This appears to be due to the release of VEGF,  $\beta$  various mitogenic growth factors (GR), like EGF, IGF-1, PDGF, FGF, TGF- etc., from activated platelets. We recently explored the growth promoting and mitogenic potential of human platelet lysates (PL) on cultured mammalian cells, and we have shown, that PL can serve as valuable alternatives to the use of FBS in cell and tissue culture (Rauch et al., 2007). In the present study, the repertoire of adherent epithelial cell lines tested was extended to anchorage-independent Raji human lymphoma cells. PL fully supported growth and proliferation of Raji cells in suspension. We further determined the actual amount of GR in different PL batches by ELISA and ascertained the optimal steps in GR enrichment during the PL extraction process. In addition to GR, the amount of hydrocortison and of prostaglandin E<sub>2</sub>, acting as synergistic/additive catabolic stimulators in chemically-defined culture media, was found to be in sufficient quantities. In order to biochemically determine the proliferative potential of PL, the stimulation of extracellular signal-regulated MAP kinase (ERK1/2) was determined. Activation of the MAP kinase signaling pathway by GR results in specific phosphorylation of downstream kinases, like ERK1/2. Addition of PL to quiescent LLC-PK1 cultures resulted in specific phosphorylation, and thus activation, of ERK1/2 within minutes. This time course is identical with ERK1/2 activation upon addition of FBS. The data further confirm the high potential of PL as valuable substitute of FBS in mammalian cell and tissue culture.

### References

Rauch et al. (2007). *ALTEX* 24, 353.

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