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Project

Human thromobocyte concentrates as substitutes for animal-derived serum in stem cell cultures for *in vitro* toxicity testing

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The role of serum in cell culture media

The supplementation of basal culture media with animal serum – in most cases fetal bovine serum (FBS) – is common practice and is essential for cell growth and for the stimulation of proliferation ("mitogenic effect"). The major functions of serum in culture media are to provide hormones, cytokines and growth factors, carrier proteins, attachment and spreading factors, and stabilizing and detoxifying factors, e. g. protease inhibitors.

The use of animal serum in cell culture bears a number of disadvantages. The disadvantages can be seen either from a theoretical, cell biological point of view, or from ethical and animal welfare perspectives. Serum is an ill-defined medium supplement, and thus an ambiguous factor in cell culture, serum batches display quantitative and qualitative variations in their composition, and thus introduce a serious lot-to-lot variability, serum may contain different amounts of endotoxins, haemoglobin, and other adverse factors, and serum can be a potential source of microbial contaminants, such as fungi, bacteria, mycoplasma, viruses or prions. Thus, serum introduces several unknown variables into the tissue culture system.

The amount of FBS produced for the world market is estimated to approximate 500.000 litres per year. More than 1.000.000 bovine fetuses have to be harvested and these numbers are expected to increase annually. Serious ethical concerns were raised with regard to the welfare of the donor fetuses in the harvest, production and processing of FBS. Fetal blood collection involves significant manipulation of the fetus. Thus, the concerns were mainly focused on the current modes of collecting FBS that may cause suffering to the animals, in particular to fetuses. As a consequence, in terms of the 3R principle and thus to avoid/reduce harvesting of FBS from bovine fetuses, a number of strategies were developed to reduce or replace the requirement for FBS in cell culture media.

Human platelet lysates, an alternative to FBS in cell culture media

An important step in the search for serum growth factors has been the finding that the most potent mitogenic factors present in serum are derived from activated thrombocytes. In a recent research project we were able to show the capacity of human platelet lysates to replace FBS in a variety of human and animal cell culture systems. Human donor thrombocytes were activated to maximally release their α-granule content of growth factors (Fig. 1). Thus, lysates of human donor thrombocyte concentrates may become a valuable substitute for animal-derived serum in cultures of human cells and in human and animal stem cell technology.

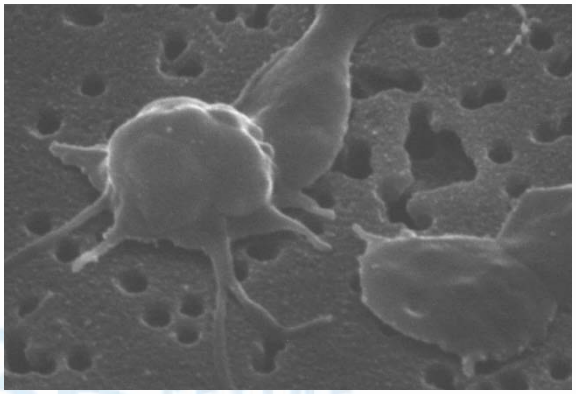

Fig. 1. Activated thrombocyte with characteristic pseudopodia.

Human Stem Cell Cultures

Originally, human embryonic stem cell lines were established by plating onto γ-irradiated or mitomycin-C treated mouse embryonic fibroblasts (MEFs) ("mouse feeder layer") in the presence of high animal serum (FBS) concentrations for derivation and propagation in an undifferentiated state. Controlling that process – either by keeping the cells in their undifferentiated state or driving specific differentiation down the desired lineage – requires careful orchestration of the culture conditions (Fig. 2).

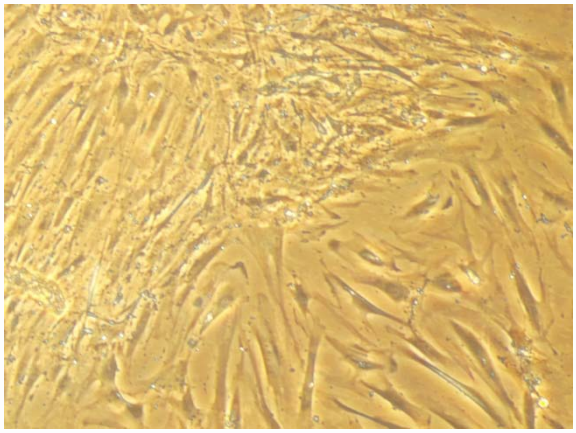

Fig. 2. Human mesenchymal stem cells in confluent culture.

However, these growth conditions for embryonic stem cells introduce the potential of transferring xenobiotic pathogens from mouse to human tissue. Recently it turned out, that both, mouse feeder layers and animal sera or animal-derived serum replacements, are sources of the nonhuman sialic acid N-glycolylneuraminic acid (Neu5Gc). Under these culture conditions, stem cells incorporate substantial amounts of Neu5Gc, against normal healthy humans have circulating antibodies. Thus, xenogenic culture methodology imperils any transplantation success, since an immune response would kill the cells *in vivo*.

Besides the high expectations in the therapeutic use of stem cells, the employment of stem cell technology for drug discovery and development or to serve as screening platforms has gained increasing importance. Human stem cell cultures, either of embryonic or adult origin, will provide new in vitro systems to detect pharmacokinetic properties and adverse toxic effects of newly designed drug compounds in innovative approaches.

Stem cell-based *in vitro* toxicity testing

Stem cell-based novel test systems are amongst the most dynamic areas of *in vitro* toxicology and biomedicine, and their development is funded e. g. by large-scale EU projects (ReProTect, www.reprotect.eu; ESNATS, www.esnats.eu; SCR&Tox, www.scrtox.eu). Stem cell technology may become the future alternative to animal testing and a key element of modern risk assessment approaches. As a prerequisite, new stem cell-based test systems have to be

elaborated and established, respectively, and their performance under animal-derived component free culture conditions has to be defined in prevalidation and validation studies. The present project - culturing stem cells under animal-derived component free conditions - should help to accomplish these tasks.

Specific aims

Lysates of human donor thrombocytes, elaborated in our laboratory, shall be used as an alternative to the high content of FBS in culturing human mesenchymal and mouse embryonic stem cells. Furthermore, stem cells cultured under these conditions shall be checked for their usability as in vitro test systems.

The following questions should be answered:

- Can stem cells of different provenance be cultured in the presence of platelet lysates?
- Under those culture conditions, can stem cells be kept in undifferentiated state?
- Can stem cells - under the above culture conditions - be triggered to differentiate into specific lineages as in the presence of FBS?
- Can the embryonic stem cell test (EST) be performed under the culture conditions with platelet lysates? Are the results comparable with cultures in FBS?

Results

The research program was successfully finalized by Dec. 31, 2013.

The initial questions

- Can stem cells of different provenance be cultured in the presence of platelet lysates?
- Under those culture conditions, can stem cells be kept in their undifferentiated state?
- Can stem cells - under the above culture conditions - be triggered to differentiate into specific lineages as in the presence of FBS?

can be answered positively.

It was shown, that adipose-derived adult human stem cells (ADSC) could be cultured in the presence of platelet lysates, and that the cells retained their undifferentiated oligopotent phenotype under these culture conditions (Rauch *et al.*, 2014a).

Furthermore, ADSC under platelet lysates also retained their full differentiation potential into mesodermal tissue lineages (Rauch *et al.*, 2014b).

Publications

The results were published in two comprehensive original articles (open access):

Rauch C., Wechselberger J., Feifel E. and Gstraunthaler G. Human Platelet Lysates Successfully Replace Fetal Bovine Serum in Adipose-derived Adult Stem Cell Culture. J. Adv. Biotechnol. Bioeng. 2(1): 1-11, 2014a.
http://www.synergypublishers.com/downloads/jabbv2n1a1/

Rauch C., Feifel E., Flörl A., Pfaller K. and Gstraunthaler G. Human Platelet Lysates Promote the Differentiation Potential of Adipose-derived Adult Stem Cell Cultures J. Adv. Biotechnol. Bioeng. 2(2): 39-48, 2014b.
http://www.synergypublishers.com/downloads/jabbv2n2a1/

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Duration

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