Semi-automated Production of Platelet Lysate

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Background:
Cell cultures for cell therapy and regenerative medicine are mainly performed in the presence of fetal bovine serum (FBS). Due to its animal origin, the use of FBS bears the risk of prion disease transmission or immunogenicity of xenogeneic proteins. Additionally, collection of FBS is highly controversial. Human platelet lysate (hPL) is known for its high growth factor (GF) content. Hence, it turned out to be a potential substitute for FBS. Since 2007 the Red Cross Blood Transfusion Service for Upper Austria in Linz deals with GMP-conform processing of hPL. Our recent aim was the development of a semi-automated process for the production of platelet lysate.

Methods:
Buffy coats that were not needed for production of platelet concentrates were used. Pools consisting of six buffy coats were centrifuged to separate platelet rich plasma (PRP) from red blood cells (RBC). A second centrifugation step was performed for the removal of surplus plasma. The platelet pellet was resuspended in 100 ml sodium chloride solution (NaCl). Samples were taken for quality control purposes before freezing the hPL units. Quality control included platelet count, pH-value, residual white and red blood cells (RBC and WBC) and platelet activation. Furthermore, samples drawn were stored at -80°C until use for GF analysis by ELISA technology (R&D Systems, UK).

Four conditions of the production process were distinguished. Separation of PRP from RBC and removal of surplus plasma was performed either manually or automatically using the Compomat G4 (Fresenius, Austria). In both cases 100 ml NaCl was added to some pools to check whether it reduces platelet losses (1= pools without NaCl; 2 = 100 ml NaCl added).

Results:
1. Platelet content, activation and recovery
Platelet recovery was best in case of automated fractionation, where no NaCl was added to the pools (=automated 1; Fig. 1+2)

2. pH levels and residual blood cells
The pH-levels were all within the set quality criteria (6.4 – 7.4) defined for thrombocyte concentrates at the Red Cross Blood Transfusion Service. Concerning residual RBC and WBC, the best results were achieved with the manual procedure without the addition of NaCl (= manual 1; see Fig. 3 and Table 1)

3. Growth factor analysis (n=4 per condition):
The highest yield in platelet derived-GF BB and epidermal GF was obtained with automated 1. Concerning insulin-like GF 1 and fibroblast GF the highest concentrations were found in manual 2 and automated 2 respectively (see table 2).

Conclusion:
The use of a bag system in combination with the Compomat G4 allows the sterile, semi-automated production of hPL. The use of sodium chloride did not lead to an improved platelet recovery and also led to an increased platelet activation. In general, further optimization should improve the results concerning residual cells enabling the implementation in routine processing.