control. Transfection efficacy was comparable, but that might not as practical to perform and requires increased amounts of medium (Table 1, run 7).

[0046] Safety Improvement by Switching of CO_2

[0047] Another surprise was seen during the large scale transfection. Transfection mix containing DNA, PEI and medium without FBS was done. Base and DO controls as well as perfusion were OFF during transfection, but CO_2 control was ON. Everything seemed to working, and the values on the screen of the iCELLis™ were as they were supposed to be. Anything else unusual was not noticed at that point. A 5 ml sample was taken from the bioreactor at 14:00 into a 15 ml Falcon tube for glucose and lactate measurements. Before the sample was taken, the tube was normally shaped. After taking the sample, the operator who took the sample was holding the tube, while emptying the sample bottle back into the bioreactor. After 2-3 min the operator viewed the tube that contained the sample and surprisingly found that the tube had changed its form. It was collapsed/flattened, but no scratches were visible. Later, also a plastic Erlenmeyer flask containing sample from the bioreactor also appeared to be "melted" as if by excess heat. The apparent "melting," however, was not caused by the heat. It was found that PEI can react with CO_2 causing a chemical reaction. Based on all what happened and what was seen, it was concluded that CO_2 flow should be shut down to be able to avoid any chemical reaction in a bioreactor. This can be a major safety aspect, too.

TABLE I

Transfection optimization and the influence in the transfection efficacy and the total yield

Run number	Transfection mix	Transfection efficiency	Yield
1	PEI and DNA 300 ng/cm^2 in 120 ml medium without serum (+glut + P/S) -> separate mixes (a'120 ml). Visual aggregation seen. Mixes in 50 ml Falcons, vortexing pairwise and pouring into glass beaker -> 15 min incubation RT without mixing	35-40%	4,05E+09
2	PEI and DNA 300 ng/cm^2 in 240 ml medium without serum (+glut + P/S) -> separate mixes (a'240 ml) Mixes in 500 ml erlenmayer -> PEI mix was poured into DNA mix -> Mixing with magnetic stirring -> 10 min incubation RT without mixing	43%	2,83E+09
3	PEI and DNA 300 ng/cm^2 in 240 ml mediumwithout serum (+glut + P/S) -> separate mixes (a'240 ml) Mixes in 500 ml erlenmayer -> PEI mix was poured into DNA mix -> Mixing strongly with magnetic stirring -> 10 min incubation RT without mixing	75%	2,09E+09
4	PEI and DNA 300 ng/cm^2 in 240 mlmedium without serum (+glut + P/S) -> separate mixes (a'240 ml) Mixes in 500 ml erlenmayer -> PEI mix was poured into DNA mix -> mixing with magnetic stirring -> 5 min incubation RT mixing gently	65-70%	3,17E+09
5	PEI and DNA 400 ng/cm^2 in 320 ml medium without serum (+glut + P/S) -> separate mixes (a'320 ml) Mixes in 500 ml and 1 liter erlenmayer -> PEI mix was poured into DNA mix -> mixing with magnetic stirring -> 7,5 min incubation RT mixing gently	60-80%	5,20E+09
6	PEI and DNA 300 ng/cm^2 in 240 ml medium without serum (+glut + P/S) -> separate mixes (a'240 ml) Mixes in 500 ml erlenmayer -> PEI mix was poured into DNA mix -> mixing with magnetic stirring -> 5 min incubation RT mixing gently	50-60%	9,06E+09
7	DNA 336 ng/cm^2 in 240 ml medium without serum (+glut + P/S) -> separate mixes (a'240 ml) Mixes in 500 ml erlenmayer -> PEI mix was poured into DNA mix -> mixing with magnetic stirring -> 5 min incubation RT mixing gently	NA	