Cord blood: spotlight on the processing step

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Cord blood (CB) can be stored processed or un-processed. The advantage of applying a processing step to the cord blood units (CBUs) for cord blood banks (CBBs) is that the volume can be reduced by depletion of plasma and sometimes also by reduction of red blood cell (RBC) number. This decreases the associated costs for storage. Another advantage is the reduction of the quantity of cryopreservation solution in the final product. As the RBCs tend to burst during the freezing and thawing step the decrease in RBC numbers reduces the possibly harmful cellular debris and free hemoglobin released by burst RBCs into the CBUs. However processing can potentially cause also a loss of the wanted cells, like the stem cell compartment or cause stress to these cells. Thus, the optimal processing system reduces volume and RBCs while at the same time leaving the wanted cells like the stem cell compartment with its hematopoietic as well as non-hematopoietic stem cells unaffected.

Hematopoietic stem cells are mainly used as a source for transplants to reconstitute the blood forming cells and immune system while non-hematopoietic stem cells can support the hematopoietic stem cells in the transplant setting but are potentially useful also for other regenerative medicine or immune modulatory purposes. Many CBBs today use a processing step instead of storing the CB un-processed just by the addition of cryopreservation solution. Different processing systems exist which often employ a centrifugation step for processing of the CBUs. In addition, we described recently a manual system, CellEffic CB, which employs a filtration step eliminating the need for centrifugation and thus any machines and its associated costs. We will highlight here recent development in the cord blood industry and how the new filtration system can contribute to this industry.

Keywords: Cord Blood; Cord Blood Banking; Processing; Filtration


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Starting in the early 90s, cord blood banking has since then increased in terms of number of CBBs and of CBUs stored worldwide. An estimated ~4.73 million CBUs are stored in public as well as private banks, with more CBUs stored in private banks [¹]. Public banks store the CBUs for principally allogeneic use applied in unrelated, but sometimes also related, patients as a cord blood transplant (CBT) for hematopoietic reconstitution while private CBBs store the CBUs for a yearly fee for the purpose of using the CB autologous or within this particular family. Also, some mixed forms of banking exist. As of 2015, more CBUs from public banks have been applied clinically than from private banks [¹]. Obtaining the CB from the cord, which in the past was treated as medical waste, after birth of the child is without harm for the infant and mother. However, it has to be noted that delayed clamping is in general recommended by
the WHO in respect to infant and maternal health and nutrition outcomes [2]. From the place of delivery, where the CB is collected by specifically trained staff, the CB is transported to the CBBs at optimally a controlled temperature - in order to avoid extreme temperature exposures - for further processing and storage until the CB is finally released for therapeutic use when needed.

Hematopoietic stem cell transplants of CBU's have been reported primarily in the allogeneic setting, among them in the related (family) as well as in the unrelated setting [3]. More CBT have been performed from 1988 to 2014 in the unrelated setting, 10110 or 92%, than in the related setting, 826 or 8%, as reported by Eurocord in 2015 without CBTs performed in the United States [4]. Within the family, hematopoietic stem cell transplantation with CB has been utilized in malignant as well as non-malignant disease [5] like in leukemia [6] or hemoglobinopathies [7] and in the unrelated setting in, among others, leukemias, lymphomas, hemoglobinopathies, and myelodysplastic syndromes [see for example 8-13]. However, the majority of unrelated CBT is rather applied in malignant diseases (5795 or 79%) than in non-malignant diseases (1530 or 21%) as published by Eurocord in 2015 [4]. A list of number of CBTs in particular diseases, where the CB has been provided by the New York Blood Center, is available by the New York Blood Center’s National Cord Blood Program [14]. In terms of other regenerative medicine and immune modulation purposes, CB and CB-derived cells are under intense preclinical investigation for the use in stroke, limb ischemia and myocardial regeneration [15 and references herein], type I diabetes [16, 17], and foot disease in type II diabetes [18]. In addition, also some clinical data exists today, for example a clinical trial using allogeneic cord blood in combination with recombinant human erythropoietin, which by itself has neurotrophic effects, has shown improvement in motor and cognitive dysfunction in children with cerebral palsy [19]. Furthermore, a report of application of autologous CB in conjunction with Granulocyte Colony Stimulating Factor (G-CSF) in two toddlers with cerebral palsy was found to be safe, feasible and to display some functional improvement [20]. We will subsequently focus on recent findings of autologous CB and its cells, which have been banked or not, in regenerative medicine for non-hematopoietic applications. Feasibility [21-23] and safety [22, 23] with indication of potential benefits [23] of autologous cord blood cells has been shown in brain disease/disorder. Also a report on the application of autologous CB in a case of cerebral palsy was published and the observed functional neurologic regeneration in this case [24] was associated with the fact that mononuclear cells (MNCs) derived from human CB can migrate to lesioned region in the brain in a rat model of perinatal cerebral hypoxia-ischemia [25, 26]. Another preclinical report testing human CB in a rabbit model of cerebral palsy suggests that the observed beneficial effects might be due to paracrine signaling rather than cellular integration [27]. These different findings might be explained by the different delivery routes, intraperitoneal versus intravenous injection, of the cells in these preclinical experiments. In 2015, a larger clinical trial using autologous CB in children with cerebral palsy found a strong correlation between increase in white matter connectivity and functional improvement [28]. However, the contribution of the treatment with CB to the functional improvement was not analyzed in this work. Another case report of CB-derived cells in an autologous setting, namely in regenerative therapy for left heart syndrome, mentions a progressive improvement in the right ventricular ejection fraction [29]. Also in this case the contribution of the cell therapy to the observed beneficial effect has yet to be determined. Taken together it needs to be noted that there is mainly preclinical data available and only little published clinical data for efficacy of autologous CB and its cells in regenerative medicine, while it appears feasible and safe. The community will watch closely for further development in this area.

The CB stored in the CBB, until applied as either for CBT of hematopoietic stem cells or for regenerative medicine / immune modulation purpose, needs to go through a series of steps, each one potentially causing stress to the cells. In brief, the CB is first collected, then it is transported to the CBB. Third, it is processed or stored un-processed. Addition of cryopreservation solution is the forth step. Then, CB is stored in the vapor phase of liquid nitrogen, after having been cooled down by a controlled rate freezer, for an extended time until it is needed. Next, the CB is transported to and received by, still frozen below -150°C, the transplant center [30]. Last but not least, the CB is thawed which might include a washing step and applied. At different steps the CB is tested for a series of markers and potency. While the cord blood cells can potentially be compromised at all of these steps, the remaining part of our research highlight will focus on the processing step of the CBU.

Different processing systems exist today. Many of them are based on centrifugal force and employ one kind of sedimentation agent or another in order to reduce RBCs efficiently. We have shown for a new kind of processing system, which is based on a filter and doesn't require centrifugation, that it works equally well with a sedimentation agent [hydroxyethyl starch (HES) solution] as well as without any sedimentation agent just by the use of physiological saline solution in terms of reduction of RBCs and recovery post-processing of total nucleated cells (TNCs), MNCs as well as CD34+ cells [31]. This is noteworthy as another processing system has been reported to have
comparably lower RBC reduction without HES \cite{31, 32} than a third report which shows for the same processing system a higher RBC reduction when used with HES \cite{33}. As the filter-based device for processing of CBUs, CellEffic CB, is new on the market it was important for us to show that reduction of RBC numbers post-processing was not due to damage of RBCs by the whole processing procedure with the device. This was done by measuring plasma-free hemoglobin levels (N. Sato, unpublished data).

Impact of higher number of RBCs on CBUs has to be determined, however more cellular debris and free hemoglobin from burst RBCs will be released into the CBU after freezing and thawing. In bone marrow transplant setting it was suggested to minimize the hemolysate content of cryopreserved bone marrow as this was associated with acute renal failure \cite{34}. In the extreme case of not removing RBCs, the use of RBC-replete, unwashed CBUs has been associated with severe or even life-threatening infusion-related adverse events \cite{35}. Thus, a RBC-replete CBU should ideally be washed, however RBC-replete CBUs have been reported to be more complicated to wash as the “…cellular debris and free haemoglobin can interfere with the demarcation of the interface between mononuclear cells and the supernatant, and can contribute to viscosity and clumping.” \cite{35}. Based on this we conclude that RBCs should be removed to a low level of contaminating cells before freezing and thawing by processing of the CBU. Furthermore, reduction of the volume of the CBU stored by processing the CBU leads to a reduction of the volume of cryopreservation solution in the final product \cite{36}.

Additionally, processing the CBU usually also removes the plasma and this will result in even smaller CBUs to be stored and thus reduces the storage costs. These latter ones consist not only of liquid nitrogen consumption but also of storage space including the liquid nitrogen tanks needed as well as the space for the liquid nitrogen tanks, and electricity. Since early on in the history of cord blood banking, the processing of CBUs was seen as a solution to reduce the storage space needed for the frozen CB samples \cite{37}. However, care has to be taken not to remove too much of the wanted cells during processing, like the cells which constitute the stem cell compartment. Taken together, cord blood processing is a trade-off between reducing RBCs and plasma while keeping other cells unaffected and highly recovered \cite{36}. Figure 1 shows the effect of cord blood processing in an ideal case scenario. The new filter-based device, CellEffic CB, which we described recently \cite{31}, can reduce the volume of the CBU up to 21mL minimum volume (without cryopreservation solution), which is within the range of volume of processed CB aimed often by CBBs to store as of today. Importantly, at the same time different stage hematopoietic and non-hematopoietic stem cells, which are useful for hematopoietic reconstitution and potentially for other regenerative medicine / immune modulatory purposes, can be recovered with CellEffic CB \cite{31}.

To our knowledge, for most clinicians today, the TNC count in the processed CBU is the most influencing factor –
next to human leukocyte antigen (HLA) matching - to decide which of the CBUs to use as CBT in the unrelated setting. This is consistent with a report on how unrelated CBUs for CBTs are selected at the transplant service at the Memorial Sloan-Kettering Cancer Center in New York. Additionally, a study by Bart et al. has found that units in US and Switzerland selected for transplants are rather the ones with higher TNC counts. However, other factors despite TNC count, like bank of origin, infectious disease markers, sterility, and hemoglobinopathy screening, have been reported to play a role in selecting the CBU used in the transplant setting. We especially want to point out the colony-forming unit (CFU) ability of the CBU after thawing of the stored CB. A single-center study of 435 unrelated CBUs has identified total CFUs post-thaw as strong predictor of neutrophil and platelet engraftment. We presented data that the filter-based device, CellEffic CB, is – next to good TNC viability post-processing as well as post-thaw - able to recover CFUs to an extremely high level even after freezing and thawing. We conclude that this points to a gentle processing by the filter, which consists of nonchemical coated and non-woven polyester fabric, as the cord blood processing step is driven by gravity only without applying centrifugal force to the cells. As CFU assay requires two weeks to be performed, a more rapid CB potency assay which determines aldehyde dehydrogenase bright (ALDHBR) content found to be correlated with CFU content, might be useful after further validation as an alternative to CFU assay post-thaw for prediction of the ability of the CBU to engraft. A recently reported decrease in CBT in absolute numbers might be an indicator for the need to store more high quality CBU based on the potency of the CBU post-thaw.

To date, multiple cord blood processing devices and methods exist which have been analyzed in terms of cell recovery and depletion in different compositions. Some CBBs prefer automated systems, which can be explained by certain advantages associated with automation, but to our knowledge this is rarely based on systematical analysis for contribution of recovered cells and influence of contaminating RBCs retrieved by different processing methods in the clinics. Also manual methods are used today and we believe that clinical data and/or data from animal models for the different processing methods need to be compared and attributed to differences in cell composition and potency post-process and, in the cases where the CB is stored before usage, post-thaw in order to decide on the optimal processing system. Identifying the optimal processing system might also help to overcome some of the limitations of CBT, like the reported poor immune cell reconstitution and delayed engraftment.

### Conflicting interests

Kaneka Corporation is manufacturer of CellEffic CB and Kaneka Pharma Europe N.V. is the European authorized representative and distributor for CellEffic CB. The work has been financed by Kaneka Corporation.

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### Author contributions

NS designed, conducted the work and analyzed the results. NS, CF reviewed and interpreted the data. CF wrote the manuscript. All authors read and approved the final manuscript.

### Abbreviation

ALDHBR: aldehyde dehydrogenase bright; CB: cord blood; CBB: cord blood bank; CBT: cord blood transplant; CBU: cord blood unit; CFU: colony-forming unit; G-CSF: Granulocyte Colony Stimulating Factor; HES: hydroxyethyl starch; HLA: human leukocyte antigen; MNC: mononuclear cell; RBC: red blood cell; TNC: total nucleated cell.

### References

2. WHO [http://www.who.int/elena/titles/cord_clamping/en/]
9. Bradstock KF, Hertzberg MS, Kerridge IH, Svennilson J,


37. Sousa T, de Sousa ME, Godinho MI, Mendes C, Carvalhais A, Barbosa IL. Umbilical cord blood processing: volume reduction and recovery of CD34+ cells. Bone Marrow Transplant 1997;


