Advances and challenges in umbilical cord blood and tissue bioprocessing: procurement and storage

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Umbilical cord tissue and blood is banked to complement the rapidly advancing field of tissue engineering and regenerative medicine for both autologous and allogeneic therapeutic applications. Whilst many problems concerning the use of the hematopoietic and multipotential mesenchymal stromal cells contained therein may be addressed through the future development of GMP-compliant manufacturing strategies, collection and bioprocessing of these tissues can be optimised in the present to maximise clinical outcomes. In this review, we describe current procurement, processing and storage approaches for umbilical cord blood and tissue; current challenges and how these may be met to augment translation and use of therapeutics harnessing their derivatives.

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Cord blood and tissue banking is increasingly popular on account of the stem cells they contain and the clinical research associated with such material advances. Umbilical cord blood (UCB) is a source of hematopoietic stem cells (HSCs; hereafter referred to as UCB-HSC) and is clinically applied for the reconstitution of hematopoiesis [1,2]. Umbilical cord tissue (UCT) contains mesenchymal stem cells (hereafter referred to as UCT-MSC) that have been widely investigated for applications in tissue restoration and repair, and the treatment of immune-mediated disorders [3,4]. UCB is defined as the blood that
remains in the umbilical cord and placenta following neonatal delivery, and UCT as the cord itself. Both tissues are procured immediately after birth in a process not deleterious to mother or child. In fact, banking of these tissues may confer future health benefits to the corresponding child or immediate family as they potentially comprise an exact or partial human leukocyte antigen (HLA) match, reducing complications such as graft-versus-host disease (GvHD) upon transplantation. Additionally, both tissues may be applied in allogeneic models; UCT-hMSC are immune evasive and donor-recipient mismatches at some HLA loci are well tolerated in UCB transplantation [5].

The potential applications of UCB-HSC and UCT-MSC in both autologous and allogeneic therapeutics has encouraged the coexistence, sometimes within the same organization, of two tissue storage models [6]. Parents may choose to publicly bank their UCB for use for any patient, or store privately in the event of disease in the immediate family for which UCB transplantation is indicated. Over 750,000 UCB units are thought to be banked publically worldwide, with over 4 million banked in private family storage arrangements [7]. No storage data is available for UCT, in part because UCT-MSC has no current clinical application, and consequently is not publicly banked. There are, however, a considerable number of clinical trials concerning the application of UCT-MSC, creating demand for UCT processing strategies that main the viability, efficacy and safety of any derived therapeutic (Table 1). In this review, we offer insight into how the tissue bioprocessing of today may be tailored to ensure the safety and efficacy of the UCB- and UCT-derived cell therapies of the future.

UMBILICAL CORD BLOOD BIOPROCESSING

Transplantation of HSC is an established treatment for reconstitution of hematopoiesis in numerous disease states [2]. These include myelodysplastic syndromes [8,9], leukemias [10,11], lymphomas [12], multiple sclerosis [13], metabolic diseases [14] and numerous disorders of blood cell proliferation, including sickle cell disease and Fanconi’s anemia (Table 2) [1,15]. UCB provides a viable alternative to other source tissues, including peripheral blood (PB) and bone marrow (BM) [16]. Although most HSC transplantations are currently sourced from PB, challenges persist that may be resolved through the use of UCB. For example, HSC extraction from PB by apheresis after administration of hematopoietic growth factors has known side effects in pediatric donors including perivascular pain, emesis, hypotension, urticaria, numbness, chest pain, facial flushing and hypocalcemia. The lower intracorporeal blood volume of children is believed to result in this reduced safety profile, with a complication rate of 6% recently reported in such patients [17,18]. Significant immune hemolysis upon transplantation has also been reported as a result of minor ABO blood group system incompatibility between donor and recipient [19]. Additionally, the long duration of apheresis (~4 hours), which must be supervised by a health care professional (HCP), increases healthcare costs.
## TABLE 1

Summary of the first 15 open clinical trials, sorted by relevance, found by searching ‘umbilical mesenchymal stem cells’ as an intervention at www.clinicaltrials.gov at the time of writing (n = 40).

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Donor type</th>
<th>Phase</th>
<th>Overview</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>Allogeneic</td>
<td>Phase I/II</td>
<td>Safety and early efficacy study of intra-nasal administration of MSC trophic factors</td>
<td>NCT02192736</td>
</tr>
<tr>
<td>Autism spectrum disorder (ASD)</td>
<td>Allogeneic</td>
<td>Phase I</td>
<td>Safety study of administration of UCT-MSC every 2 months in children with ASD</td>
<td>NCT03099239</td>
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<td>Multiple sclerosis</td>
<td>Allogeneic</td>
<td>Phase I/II</td>
<td>Safety and early efficacy study of administration of UCT-MSC every 3 months for 1 year in children with ASD</td>
<td>NCT02192749</td>
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<td>Allogeneic</td>
<td>Phase I/II</td>
<td>Safety and early efficacy study of effect of administration of UCT-MSC daily for 7 days in MS patients</td>
<td>NCT02034188</td>
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<td>Rheumatoid arthritis</td>
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<td>Phase I/II</td>
<td>Safety and early efficacy study on the effect of treatment with UCT-MSC daily for 5 days to induce remission</td>
<td>NCT01985464</td>
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<td>Rheumatoid arthritis</td>
<td>Allogeneic</td>
<td>Phase I</td>
<td>Safety and early efficacy study of administration of UCT-MSC weekly for 4 weeks</td>
<td>NCT02643823</td>
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<td>Aplastic anemia</td>
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<td>Phase I</td>
<td>Safety and early efficacy study of UCT-MSC administration</td>
<td>NCT03055078</td>
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<tr>
<td>Hepatic cirrhosis</td>
<td>Allogeneic</td>
<td>Phase I</td>
<td>Safety and early efficacy study of weekly UCT-MSC administration for 4 weeks</td>
<td>NCT02652351</td>
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<td>Spinal cord injury</td>
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<td>Phase III</td>
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<td>Phase I</td>
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<td>Type 1 diabetes</td>
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<td>Phase III</td>
<td>Efficacy study of UCT-MSC treatment of patients with Type 1 diabetes</td>
<td>NCT02763423</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>Allogeneic</td>
<td>Phase II</td>
<td>Efficacy study of administration of UCT-MSC</td>
<td>NCT02633163</td>
</tr>
<tr>
<td>Nonunion fracture</td>
<td>Allogeneic</td>
<td>Early Phase I</td>
<td>Comparative study of treatment of non-union fractures with UCT-, bone marrow- or adipose tissue derived MSC</td>
<td>NCT02307435</td>
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## TABLE 2
Summary of the first 15 open clinical trials, sorted by relevance, found by searching for ‘umbilical cord blood’ as an intervention at www.clinicaltrials.gov at the time of writing (n = 139).

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Donor type</th>
<th>Phase</th>
<th>Overview</th>
<th>Identifier</th>
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<td>Cerebral palsy</td>
<td>Allogeneic</td>
<td>Not provided</td>
<td>Efficacy study of UCB administration in children with CP under non-myelo-</td>
<td>NCT01639404</td>
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<td></td>
<td></td>
<td>Phase I</td>
<td>blative immunosuppression</td>
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<td></td>
<td>Autologous</td>
<td>Phase II</td>
<td>Safety study of UCB administration in children with CP</td>
<td>NCT01147653</td>
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<td></td>
<td>Allogeneic</td>
<td>Phase II</td>
<td>Safety study of sibling UCB transplant in children with CP</td>
<td>NCT02599207</td>
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<tr>
<td></td>
<td>Allogeneic</td>
<td>Phase II</td>
<td>Efficacy study of UCB administration in children with CP under non-myelo-</td>
<td>NCT01528436</td>
</tr>
<tr>
<td>Global development delay</td>
<td>Allogeneic</td>
<td>Not provided</td>
<td>Efficacy study of UCB administration in children with Global Development</td>
<td>NCT01601158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase I</td>
<td>Delay under non-myeloblastic immunosuppression</td>
<td></td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>Allogeneic</td>
<td>Phase I</td>
<td>Safety study of single UCB unit transplantation, matched by ABO/Rh, within</td>
<td>NCT02397018</td>
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<tr>
<td></td>
<td></td>
<td>Phase I</td>
<td>3-10 days of acute stroke</td>
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<td></td>
<td></td>
<td>Phase II</td>
<td>Safety study of UCB transplantation and mannitol within 72 hours of stroke</td>
<td>NCT02433509</td>
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<td></td>
<td>Phase II</td>
<td>Efficacy study of single UCB unit transplantation, matched by ABO/Rh and</td>
<td>NCT03004976</td>
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<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>ethnicity, within 3-10 days of acute stroke</td>
<td></td>
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<tr>
<td>Autism spectrum disorder</td>
<td>Autologous</td>
<td>Phase I</td>
<td>Safety study of single UCB unit transplantation in children with ASD</td>
<td>NCT02176317</td>
</tr>
<tr>
<td>Infertility</td>
<td>Allogeneic</td>
<td>Not provided</td>
<td>Efficacy study of the treatment of infertility caused by severe intrater-</td>
<td>NCT02313415</td>
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<tr>
<td></td>
<td></td>
<td>Phase I</td>
<td>inner adhesions or endometrial dysplasia by UCB derived MSCs on a collagen</td>
<td></td>
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<td></td>
<td></td>
<td>Phase I</td>
<td>scaffold</td>
<td></td>
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<tr>
<td>Congenital pediatric disorders</td>
<td>Allogeneic</td>
<td>Not provided</td>
<td>Safety study of UCB transplantation for congenital paediatric disorders, esp.</td>
<td>NCT00950846</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>wrt incidence of GvHD</td>
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<td>Immunodeficiency</td>
<td>Allogeneic</td>
<td>Phase II</td>
<td>Efficacy of intrabone infusion of UCB to improve hematopoietic reconstit-</td>
<td>NCT01711788</td>
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<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>tion</td>
<td></td>
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<tr>
<td>Hypoplastic left heart syndrome (HLHS)</td>
<td>Autologous</td>
<td>Phase I</td>
<td>Safety and feasibility study of intraventricular administration of UCB in</td>
<td>NCT01883076</td>
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<td></td>
<td></td>
<td>Phase I</td>
<td>children with HLHS</td>
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<tr>
<td>Neonatal encephalopathy</td>
<td>Autologous</td>
<td>Phase I</td>
<td>Safety and feasibility of UCB transplantation within 72 hours postpartum</td>
<td>NCT02256618</td>
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<td>Type 1 diabetes</td>
<td>Allogeneic</td>
<td>Phase I/II</td>
<td>Isolation, ex vivo expansion and transplantation of regulatory T cells from</td>
<td>NCT02932826</td>
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<td>UCB</td>
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Bone marrow, obtained in an invasive and painful procedure, has commonly been used as a source of HSC [20]. The functional characteristics of HSC are not constant throughout life; they exhibit reduced proliferative potential and myeloid-biased differentiation output with aging. This reduced capacity for lymphoid differentiation is believed to account for the decline in immune function in the elderly, and consequently HSC from aged donors administered for hematopoietic reconstitution may result in poorer patient outcomes than HSC from younger donors, such as the neonate population from which UCB-HSC are derived [21,22].

UCB offers an allogeneic source of HSC in lieu of PB and BM, and has been employed in clinical trials for the treatment of a range of indications including blood cancers, diabetes, stroke and Fanconi’s anemia (Figure 1 & Table 2). In fact, since the first successful transplant in 1988 for the treatment of Fanconi’s anemia [1], it is estimated that more than 35,000 UCB transplants have been performed worldwide [7].

Procurement, processing & storage of UCB

UCB procurement

As for most tissue donation, donors of UCB, regardless of storage model, must provide informed consent prior to collection, and undergo infectious disease screening between 7 days before and 7 days after delivery (Figure 2, stage A). Units are always procured postpartum, but may be collected before or after separation of the placenta from the uterus. These methods are termed in and ex utero procurement respectively (Figure 2, stage C) [23]. Additionally, UCB procurement is not limited by birth type; units are routinely collected following both induced and non-induced vaginal and cesarean births. For all approaches, the blood supply from the umbilical cord to the neonate is interrupted by postpartum clamping following birth, and the residual cord blood extracted by venipuncture. In the UK, the Human Tissue Authority (HTA) recommends ex utero procurement of UCB [24]; in contrast, in utero collection is widely favoured elsewhere, including the USA and Western Europe, due to the significantly greater sample volumes and total nucleated cell counts (TNCs) achieved [25]. This may be due to the fact that, during the third stage of labor, the uterus continues to contract, aiding the expulsion of blood [26].

Impact of obstetric practices on UCB quality

The final volume and total nucleated cell count (TNC) of UCB collected is partially dependent on uncontrollable obstetric factors, such as maternal age [27], gestational period [28,29], placental weight [30], number of prior live births [31,32], delivery type [33–35], maternal smoking [36], cord length [28,37], ethnicity [38], maternal pre-eclampsia [39,40], fetal distress [34], birth weight [28,41], and possibly neonate sex [27]. However, birth and procurement approaches, under clinician and maternal control, may also impact UCB unit quality. For example, the timing of cord clamping directly impacts the volume of UCB collected [42] and the pre-venipuncture preparation of the cord impacts UCB unit contamination [43,44].

Delayed cord clamping, classed as interruption of the blood supply
between cord and infant after more than one minute postpartum, is recommended by the World Health Organisation (WHO) [45]; extended placental transfusion following delivery improves term infant iron status for up to 6 months and reduces the risk of maternal postpartum hemorrhage (Figure 2, stage B) [26,46]. Additionally, delayed cord clamping reduces the risk of intraventricular hemorrhage, late-onset sepsis and necrotizing enterocolitis in pre-term neonates [47]. The loss of blood flow as a result of delayed cord clamping, temperature changes and other factors cause vascular occlusion, which in turn triggers coagulation of entrapped blood [48].

The volume of UCB units collected following delayed cord clamping are significantly reduced as a result, although some tissue banks advise that UCB collection is still viable if the cord is clamped after no more than 3 minutes. Similarly, those procured following expectant versus actively managed labors (whereby prophylactic uterotonics are administered to hasten delivery of the extraembryonic tissues) are also of smaller volume [49].

UCB is contained within the aseptic, closed system comprising baby, placenta and cord, and is drained into a sterile container for subsequent processing. Microbial contamination is therefore not present in either UCB (in the absence of vertically transmitted infections) or storage materials; in fact, contaminants may only be introduced at the point of procurement during which UCB is briefly exposed to the cord exterior. The birth environment is inherently non-sterile because, particularly during vaginal births, the epithelial amniotic membrane is in contact with vaginal and colon-derived fluids. Microbes transferred via these fluids then access the cord interior through the procurement venipuncture. The risk of microbial contamination of UCB through
venipuncture should be reduced through the use of antimicrobial surgical swabs at the puncture site prior to drainage; hence HCP compliance with tissue procurement instructions is vital in ensuring the suitability of UCB units for clinical application [43].

Transport of UCB
Following procurement, UCB units and any other samples collected are placed in transport packaging (Figure 2, stage E). During sample transit, environmental conditions critical to the maintenance of cell viability, such as the partial pressures of CO₂ and O₂, temperature and pH are largely uncontrolled and are therefore reliant on external conditions and the duration of transit. However, packaging may be optimized to both meet regulatory shipping requirements and mediate environmental changes. For example, thermally insulating materials, such as polystyrene, may be used in sample packaging, which also isolates UCB from the influence of light. Phase change materials (PCMs), that have a high latent heat capacity, incorporated within gel packs, can be placed in transport boxes, to further augment temperature buffering [50]. Additionally, temperature recording apparatus may be included in transport boxes, and samples may be transported in refrigerated vehicles or packaging to retard microbial growth in transit, and maintain cell viability [51]. Additionally, cooling of the external environment may have limited success for samples stored in thermally insulated packaging; the authors suggest that suitable ‘inside

FIGURE 2
Process map of usual UCB procurement (blue) and processing procedures (yellow).
out' cooling technologies that may be enclosed in transport boxes are lacking and should be developed. In the authors’ experience, logistical factors between the site of procurement and storage can impinge on the quality of a cord blood unit. Interestingly, private cord blood banking is prohibited in some countries in favor of public banking models, but export of samples for overseas processing and storage is sometimes permitted. On average, the procurement volume of private units originating from such countries is smaller than from other regions [Unpublished data]; we speculate that this may reflect the reservations of HCPs regarding the ethics of private cord blood banking. Furthermore, extended transit, as may be caused by geographical and local customs/legal requirements, is associated with lower white blood cell counts and HSC viability [Unpublished data].

Bioprocessing of UCB for cryopreservation

UCB processing may incorporate automated or operator-based, and whole blood or volume-reduced approaches (Figure 2, stage G onwards). Economic, safety and quality considerations inform the technologies employed in a given paradigm. A balance must be sought between the comparatively reduced storage costs and the greater processing time required for volume reduced versus whole blood cord units [52]. Additionally, volume-reduced transplants confer improved patient outcomes, such as a reduction in ABO incompatibility alloreactivity and dimethyl sulfoxide (DMSO) toxicity (Figure 2, stage H) [53].

In volume reduction protocols, the red blood cell and plasma fractions of units are largely depleted. This approach has been adopted by many banks worldwide, including public organisations such as the NHS Cord Blood Bank (UK). In summary, incoming units are separated by centrifugation or filtration and plasma, red blood cell and HSC-containing 'buffy coat' fractions are separated using either semi-automated devices such as the Macopress Smart Evo (Macopharma, France), Optipress II (Fenwal, USA) and CellEffic (Kaneka, Japan) products, or fully automated, enclosed systems (such as the Sepax™ (Biosafe, USA) or AutoXpress® (Cesca Therapeutics, USA) [49,54]. Cryoprotectant is added to theuffy coat fraction, which is stored in vapor phase N₂. Sedimentation agents such as pentastarch [55] and hydroxyethyl starch (HES) [56] may also be used to enhance red blood cell removal. Fully or semi-automated systems may offer advantages with respect to standardization, necessary to ensure the safety and efficacy of UCB-derived cell therapies, and allow UCB processing to take place in ungraded environments. In whole blood storage protocols, the complete unit is cryopreserved following cryoprotectant addition with no additional processing steps [57].

Challenges in the clinical application of UCB

Use of UCB for haematopoietic reconstitution was first reported by Ende et al., for the treatment of acute lymphoblastic leukemia, in 1972 [58]. Unfortunately, clinical use of UCB is limited by the availability of human leucocyte antigen (HLA)-matched donors, although mismatches at one or two loci are well tolerated [5]. Approximately
30% of all recipients are matched with an HLA-identical donor through worldwide registries; around 75% of Caucasians [59], but only 17% of other ethnicities find a suitable donor [60].

Low hematopoietic progenitor yields per unit present a yet more significant challenge for the clinical use of UCB, and mean that only low weight patients, such as children, are eligible for transplant of single units [61]. Low cell doses are also associated with increased engraftment times, prolonged immuno-nodeficiency and greater healthcare burdens [62]. Double UCB transplantation, an alternative treatment regimen that increases the number of HSC transplanted, has been widely reported to improve clinical outcomes [63], but is associated with greater acquisition costs. However, one notable study by Labopin et al., in which Markov analysis was used to evaluate the costs and outcomes of single and double UCB transplantation in France, reported the latter as more cost-effective [64].

Alternatively, ex vivo expansion of UCB-HSC may increase available cell doses, and manufacturing strategies to produce advanced therapy medicinal products (ATMP) are under investigation [65]. Current UCB therapies are considered minimally manipulated products by regulators [66,67].

**UMBILICAL CORD TISSUE BIOPROCESSING**

UCT is banked to preserve UCT-MSC, which offer different clinical potentials to UCB-HSC. The identification of clonogenic fibroblast-like cells in bone marrow by Friedenstein et al. in 1970 [68] is oft viewed as the dawn of modern MSC research. MSC have subsequently been widely investigated for applications in wound healing and various pathologies, utilizing their multilineage plasticity and immunomodulatory functions [5,69]; at the time of writing this review, there are approximately 2600 clinical trials involving MSC, of which 40 utilized UCT-derived MSC (www.clinicaltrials.gov; search terms: “mesen*” AND “cell”, “MSC”, “umb*” AND “mesen*”). Although MSC reside in virtually all postnatal tissues [70], they have been frequently sourced from bone marrow. Major drawbacks associated with bone marrow sources include the invasiveness of aspiration, influence of donor age on the functional characteristics of MSC and their paucity within this tissue. In comparison, UCT is considered clinical waste, is relatively MSC dense, and is sourced from neonates [71]. Other clinically useful properties of MSC include the lack of expression of MHC-II (major histocompatibility complex II) and co-stimulatory molecules CD80 and CD86, conferring hypoimmunogenicity. Hence, MSC derived from suitable sources and subjected to appropriate bioprocessing may also be harnessed for ‘off-the-shelf’ allogeneic therapeutics [72].

**Challenges in procurement & storage of UCT**

UCT is procured following neonate delivery and transported for processing in transport solution. Following neonate delivery and UCB procurement the cord is severed from the placenta, disinfected, and placed in a sterile container (Figure 3, stages A–E). Tissue viability and sterility are impacted by
the same transit, packaging and environmental factors as described previously for UCB (Figure 3, stage F). Upon arrival at a processing laboratory, cord tissue processing is typically performed using the ‘slice and dice’ method (Figure 3, stage J) [73]; briefly, UCT is disinfected, rinsed, minced and added to cryoprotectant before controlled rate freezing and long-term storage in vapor-phase N₂ (Figure 3, stages G–M). Although tissue procurement is relatively uncontrolled due to the clinical demands of the birth environment, the authors recognize that key aspects of industry practice may be improved to optimize UCT collection and storage [74].

Microbial contamination of UCT

The quality of banked cord tissue may be considered in terms of sterility, cell yield, functionality and anticipated safety for clinical application. Since UCT is maintained in aseptic conditions throughout transit and processing, microbial contamination may only be introduced at the point of procurement as a result of contact with the ex utero environment. The collection method used may influence the probability of microbial contamination; we have found that in utero procurement can be associated with UCT contamination rates approximately three times greater than ex utero counterparts [Unpublished data]. Additionally, microbial species found on in utero procured samples are overwhelmingly of faecal origin; this is likely due to extended proximity to excreta in the birth environment [Unpublished data]. The course, type, and duration of birth may be reasonably
expected to affect the probability of microbial contamination but are not controllable to the UCT procurer. However, the impact of these factors and hence contact with bodily fluids may be mitigated by thorough disinfection of all samples using surgical swabs (Figure 3, stage D).

Additionally, we have also found that there may be a significant relationship between sample transit duration and the incidence of contaminated cord tissue (Figure 3, stage F) [Unpublished data]. It is postulated that this may be explained by increased microbial growth with increasing transit times. Longer incubation times may also encourage the formation of biofilm communities that are not easily subsequently eliminated; biofilms are often recalcitrant to the antibiotic and biocidal measures fatal to planktonic bacteria [75].

Yield & functionality of MSC content

The yield and functionality of MSC subsequently isolated from UCT is affected by tissue bioprocessing methods. For example, intra- and inter-cord variability in MSC content should be considered with respect to tissue procurement. An optimal cell donor cannot be selected in autologous paradigms, but strategic tissue sampling is possible. Lim et al. investigated the yield and functionality of UCT-hMSC isolated from tissue segments excised from distinct cord regions. Fetal and maternal segment-derived MSC had greater viability, lower doubling times and higher differentiation potentials than middle cord-derived MSC [76]. Hence, selective tissue resection from the ends of umbilical cords may improve UCT-hMSC characteristics, but further evidence is necessary to support any change in collection practices.

In allogeneic models, donor MSC are expanded for off-the-shelf supply, and both inter- and intra-cord sampling comprise controllable variables. Allogeneic donor criteria must incorporate infectious disease screening and demographic factors; studies concerning the impact of donor characteristics are limited, but reports indicate that gestational diabetes mellitus and maternal age of >29 years impact the functional characteristics of UCT-hMSC, and hence should form exclusion criteria [77,78].

Safety considerations in bioprocessing of UCT

The cryoprotectants and choice of transport/wash solutions used for UCT bioprocessing directly contribute to patient outcomes. For example, UCT is often transported in penicillin-containing solutions despite the well-documented allergenicity of β-lactam antibiotics. In fact, penicillin allergy affects up to 8% of patients, and complications can be fatal [79]. In the opinion of the authors, the use of antibiotic-treated tissues in cell therapy manufacture will present increased challenges in gaining regulatory approval.

Additionally, both cryoprotectant and cryopreservation media choice pose safety concerns. The most commonly used cryoprotectant, dimethyl sulfoxide (DMSO), is toxic at the molar concentrations used and at temperatures greater than 4°C [71,80]. In fact, a plethora of adverse reactions have been reported from DMSO exposure in humans and other mammals, including some following post-thaw infusion of
washed cells in clinical trials, where up to 50% of patients were affected [81,82]. The allo- or xeno-geneic components of serum-containing cryopreservation media, believed to supply nutrients in the post-thaw period, are notoriously difficult to remove and may trigger immunogenic responses [83].

Challenges in isolation of MSC from UCT

UCT banking has the potential to meet clinical and commercial MSC demands with the maturation of bioprocessing and expansion technologies. Unlike the UCB-HSC, UCT-MSC must always be expanded ex vivo to supply clinically relevant cell doses. Although a critical area for research and development activities, the scope of this review comprises current progress and challenges in the procurement and processing of UCT for cryopreservation. Readers are referred to the following reviews for insight regarding the ex vivo expansion of MSC [84–86].

Isolation of MSC from UCT has been described in a number of publications [87–89], but there is no consensus regarding an optimal approach that maximizes cell yield, viability and population homogeneity. UCT-MSC are isolated either by enzymatic disaggregation of cord tissue or the selective migration of MSC from cultured explants. Collagenase, hyaluronidase and trypsin are commonly used in digestion protocols [90,91], either alone or in combination, and different explant sizes and explant-substrate adhesion modes have been investigated [92]. Only one report has emerged in which a combination of both methods was investigated [93].

MSC are found throughout UCT, which may be stored as whole minced tissue, with a lack of consensus amongst investigators as to whether UCT-MSC isolated from different anatomical regions are equivalent [94,95]. However, MSC are most commonly isolated from Wharton’s jelly or the perivascular region [96,97]. Although these anatomical regions contain approximately 75% of UCT-MSC and this approach may reduce the number of contaminating epithelial and endothelial cells in early passage UCT-MSC cultures, incorporating dissection of an inherently variable tissue into a closed, automated process according to best practice, remains problematic [98,99]. Since the macroscale dimensions of individual cords differ, separation of distinct tissues must be conducted by a skilled operator, and to meet cGMP standards, manual bioprocessing must be housed in a class A biosafety cabinet installed in a Class B room [66,100].

Preservation, Shipping & Contamination Management of UCB & UCT

Contamination management strategies in UCB and UCT banking must be devised with care to maximize the clinical utility of these tissues. We propose that two main approaches should be employed to reduce the incidence of contamination: management of HCP practices to avoid initial microbial inoculation of tissue, and ablation of microbial growth during transit.

Owing to the inflated contamination rates observed in in utero procured UCT, ex utero procurement of these tissues is recommended to ensure the safety of derived UCT-MSC. Alternatively, if HCPs strongly favor...
the in utero collection method, the
sample should be taken to a des-
ignated clean area after UCB pro-
curement for thorough disinfection
according to the processing facility's
protocol, which should incorporate
a combined disinfection approach
such as treatment with both chlor-
hexidine and alcohol, rather than al-
cohol alone, since this combination
results in greater microbial kill [101].

Whilst the formation of biofilms on
both in and ex vivo soft tissues is
widely acknowledged in microbiol-
ogy, to the authors' knowledge, no
investigators have attempted to de-
tect biofilm matrix on contaminated
UCT to date. Such results could be
instrumental in refining current in-
dustry practices.

Penicillin-containing solutions
have frequently been added to tis-
sue transport solutions to ablate
bacterial growth during transit.
Whilst the impact of growth during
transit is acknowledged to be large,
penicillin is a common allergen and
treated tissue is unlikely to find al-
logeneic uses. The most frequently
encountered contaminant species
on UCT and UCB are mesophilic,
and moderate cooling during tran-
sit is suggested to extend population
doubling times as an alternative
[102,103]. The increased expense
of courier services using refrigerated
versus ambient vehicles means that
refrigeration inside sample pack-
aging is more economically viable.
This may be achieved through add-
ition of cooling devices to sample
packaging in conjunction with ther-
ally insulating packaging.

Other safety concerns impact-
ing the utility of UCB and UCT in
the clinic include cryopreservation
strategies, and whole versus volume
-reduced bioprocessing of UCB.
The success of cryopreservation
is dependent on cryopreservation
medium and the method of cool-
ing. Controlled rate freezing of
DMSO-infused tissues is preferred
throughout the tissue banking in-
dustry; vitrification of UCT has
previously been attempted to the
detriment of the UCT-MSC con-
tained within [104]. The toxicity
doing UCT to both cells and man
means that the lowest effective con-
centration should be used and post-
thaw recovery optimized. Concen-
trations of 10% (v/v) DMSO are
often used, but increasing evidence
suggests that this can be reduced to
2.5% (v/v), with benefits for the
functional characteristics of UCB-
HSC and UCT-MSC, and reduc-
ing the risk of infusional toxicity of
DMSO [105,106]. Current practic-
es achieve DMSO removal by wash-
ing with isotonic salt solutions and
centrifugation, or using automated
cell washing devices [107], but these
introduce mechanical and osmotic
stresses that can result in significant
cell loss [71].

Slow-cooling cell injury can be
further precluded through inclusion
of polysaccharides such as trehalose
[108], sugar alcohols, hydroxyeth-
yl starch (HES) [109] and dextran
in cryopreservation media [110].
DMSO readily permeates cell mem-
branes and impedes extracellular ice
formation by depressing the freezing
point of water, and polysaccharides
are thought to assist by confound-
ing the formation of intracellular ice
[111]. The addition of polysaccharide
solutions also reduces the concen-
tration of DMSO necessary for suc-
cessful cryopreservation [106,112].
Hence, we recommend that the
minimum concentration of DMSO
required, validated with respect to
optimal post-thaw cell recovery,
in conjunction with the nontoxic
additives previously listed, be used to cryopreserve UCT and UCB for translation/clinical application. Although UCT may be stored in a saline/DMSO solution, serum-containing culture media are also widely used in cryopreservation media. Although the Federal Drug Administration (FDA) (USA) permits cell therapy manufacture with cGMP-compliant sera [113], xeno- and allogeneic antigens present may be hyperimmunogenic to patients, and zoonotic, viral and prion transmission is possible. Chemically defined, serum-free medium (SFM) minimizes lot-to-lot variability in cell products, but culture substrates coated with exogenous proteins are frequently needed to aid adherence. Fortunately, successful xeno- and serum-free MSC culture has now been reported, and should be harnessed in cryopreservation media for tissue banking [114].

Volume reduction of UCB is commonplace because it reduces per unit storage costs and improves transplantation outcomes. At present, there are no known clinical uses for the RBC fraction of cord blood, and RBC lysate generated during cryopreservation and thawing can impact kidney function in recipients [61]. Alloreactivity of whole blood UCB units may also present where donor and recipient have differing blood groups, regardless of HLA type [115]. Consequently, volume reduction is recommended in UCB processing for maximal transplant success.

**TRANSLATIONAL INSIGHT**

Ultimately, effective bioprocess development is dependent upon an intimate knowledge of critical quality attributes (CQAs) of the final product and intended clinical application. Transplantation of UCB-MSC is a standard therapy for numerous diseases, but no UCT-MSC therapeutics currently exist. This is reflected in the fact that, at the time of writing, there are approximately 139 active clinical trials of UCB interventions, but just 40 employing UCT-MSC (Figures 1 & 4). Despite this, UCT-MSCs are being investigated as a therapy in clinical indications such as GvHD, hepatic cirrhosis, myocardial infarction and myopathies, spinal cord injury, arthritis, multiple sclerosis, and stroke [71,116]. A ‘chicken and egg’ paradigm exists in cell therapy research – bioprocess strategies are required to facilitate ATMP development, but clinical direction is needed to define CQAs of such bioprocesses. Clinical trials are suggested as the best indicators of clinically important parameters in tissue banking of UCB and UCT (Tables 1 & 2).

In the absence of scalable manufacturing technology, the demand for high UCT-MSC and UCB-HSC doses can be tackled through the adoption of optimal bioprocess and clinical approaches that seek to preserve both maximal cell numbers and engraftment potentials. The number of HSC administered has been increased using the scale out approach of double UCB transplantation, maintaining the ‘minimally manipulated’ regulatory status of the treatment [117]. Reduced intensity patient conditioning prior to transplantation [118,119], direct intra-bone injection [120,121] and co-transplantation of UCB with MSC for their immunoprotective function [122,123], have all been utilized to maximize engraftment. We suggest that the number of UCT-hMSC sampled during
UCT collection may be optimized through selective procurement, where practicable, of equal sized tissue segments from the fetal and placental ends of the cord.

A consensus regarding an optimal method for the isolation of UCT-MSC is yet to emerge, with scant data available to assess the merits of explant and enzymatic digestion methods. Thus, comparison studies of UCT-MSC extracted by each method are contrasted, according to their kinetics and the defining criteria outlined by the International Society for Cellular Therapy (ISCT) are required for effective translation [124].

**CONCLUSION**

To complement the body of work that aims to elucidate clinical uses of UCB and UCT-MSC, bioprocessing strategies that are optimal with respect to cell functionality, patient outcomes and cost must be developed. In this review, we argue that UCB volume reduction, antibiotic-free bioprocesses, low DMSO in cryopreservation media, and improved antimicrobial strategies may aid the readiness of UCB and UCT banked in the present for incorporation into the cell therapeutics of the future.

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