Breast milk cell components and its beneficial effects on neonates: need for breast milk cell banking

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Abstract

Universal breastfeeding has been a stated policy of the American Academy of Pediatrics, the World Health Organization as well as UNICEF. Human milk is considered as the gold standard for infants owing to its colossal nutritional values. However, the presence of various cellular components of breast milk have been gaining more attention in recent years since the first discovery of mammary stem cells in 2007, thereby providing a ray of hope not only for growth and immunity of the neonate but also an insight into its regenerative applicability. In this relation, this article summarizes the cell components of breast milk that have been identified to date. It highlights the beneficial effects of these cells for term and preterm delivered infants along with the need for breast milk and its cell banking.

Keywords

Breast milk, preterm and term infants, stem cells, cell constituents, growth factors, breast milk banking.

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Introduction

Breast milk is produced naturally by women and provides the basic nutrition for a baby for several months of life. There are three distinct stages of breast milk: colostrum, transitional milk and mature milk. Colostrum is the first fluid produced in the postpartum period. It is a dynamic fluid with a plethora of proteins, fat-soluble vitamins, minerals, immunoglobulins, various cellular components and growth factors. It lasts for approximately 2 weeks, after which mature milk starts, which lasts until milk ceases. With 90% of breast milk being water, 10% consists of carbohydrates, proteins and fats that provide growth and energy. Thus, all stages of milk provide nutritional value to the infants and hence breastfeeding is emphasized by WHO and UNICEF [1]. Apart from these nutritional components, breast milk also encompasses various cellular components, which have recently been identified by several investigators [2-7]. These complex mixtures of interacting components of breast milk contribute to the beneficial effects of breastfeeding.

The breast is composed of two types of epithelial cells. The inner or luminal cells, which are potential milk secreting cells, are surrounded by an outer basal layer of contractile myoepithelial cells [16]. It has been hypothesized that the epithelial cells are shed from the ductal and luminal epithelial layers through either a heightened turnover of the secretory tissue, or as a consequence of the mechanical shear forces associated with the continued filling and emptying cycle accompanying breast milk synthesis and lactation [4]. Prolactin acts on luminal epithelial cells and oxytocin acts on myoepithelial cells for the production and ejection of milk respectively [17].

The existence of a putative mammary stem cell population that forms the basis of these aforesaid epithelial cells and its lineage has been reported in breast milk with the expression of markers such as CK5, CK14, CK19, nestin and so on [2, 4]. Besides, human breast milk has also been identified as possessing heterogeneous cellular components such as mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), side population (SP) and endothelial cells and so on [3-5]. Furthermore, breast milk has been demonstrated to possess pluripotent properties with the evidence of the presence of pluripotent markers such as OCT4, SOX2, NANOG and their multipotent differentiation ability [6, 18].

Hence, it is believed that besides nutritional components, breast milk also possesses various stem cell and non-stem cell components. These cellular components have colossal potency for the treatment of a wide horizon of neonatal diseases. Nonetheless, there is little evidence on the applications of stem cell constituents of breast milk. It is imperative to explore these constituents, including stem cells, to open new avenues in a child’s development and regeneration. With advanced techniques, a wide spectrum of various cellular hierarchies have been identified in breast milk, from stem cells to progenitor cells to more differentiated lactocytes and myoepithelial cells [7, 19, 20]. The components of breast milk are highlighted (Fig. 1).

Components of breast milk

Breast milk is the secretory product of the mammary gland in the postpartum period. It is an undeniably unique, natural source of nutrition for the human infant. It is a dynamic fluid composed of macro- and micronutrients and other bioactive factors especially suited to meet the needs of newborn infants for their growth and development [8-11]. It possesses a complex mixture of interacting compounds such as proteins, antibodies, vitamins, growth factors, hormones, cytokines and several immunizing factors for the newborn [10, 12-14]. Accumulating evidence suggests that breast milk encompasses epithelial cells, colostral corpuscles, polymorphonuclear leukocytes, mononuclear phagocytes and lymphocytes, with those of epithelial lineage forming the main bulk of cells within two weeks of establishing lactation [10, 15]. These complex mixtures of interacting components of breast milk contribute to the beneficial effects of breastfeeding.
Mammary stem cells and epithelial cell components

Mammary stem cell research has gained interest in recent decades. Recently, enrichment of human breast stem/progenitor cells as non-adherent mammospheres in serum-free media has been demonstrated [21]. Its characterization has been simplified by means of flow cytometric techniques and spheroid formation by in-vitro culture. These different approaches have led to the successful isolation of mammary stem cells through a combination of different cell-surface markers (CD44, CD29, CD49f, EpCAM, and CD24) that generate the two main mammary cell lineages (luminal and myoepithelial lineages), producing an increasing number of cells with distinct properties. Besides, the combination of different cell-surface markers that appear to be enriched for a cell type that can repopulate the mammary gland in vivo has been delineated [20, 21]. With these advancements in mammary stem cell research, a lineage hierarchy of breast stem/progenitor cells has been identified with a view to finding the cellular origin of breast cancer and tumor heterogeneity and discovering novel drug targets for cancer treatment [22]. However, there are intricacies in isolation and culture of stem cells from mammary tissue.

Although stem cells from breast tissue have been well characterized, breast milk has recently been isolated as a non-invasive source of mammary stem cells. Several investigators have represented breast milk as a unique source of mammary stem cell population that has been exfoliated from mammary glands during ejection, with first evidence of nestin positive putative mammary stem cells [2] coupled with other markers such as CK5, CK14, CK18, CK19 and so on. This is also supported by studies by Fan et al. [4]. These mammary stem cells are hypothesized to be of epithelial origin and are considered to be present in milk through suckling force from the breast tissue.

Hematopoietic/mesenchymal and other stem/progenitor cell constituents

Breast milk was believed to possess heterogeneous cellular components such as MSCs, HSCs, SP cells, cell adhesion molecules, pluripotent cells and so on. CD44, CD29, SCA-1 positive cells along with cytoskeletal protein markers such as vimentin, smooth muscle actin and e-cadherin, an epithelial-mesenchymal transition marker, were reported [3]. Following this, Fan and co-workers also showed the positive expression of various primitive stem/progenitor cell markers.
such as CD133, Stro-1, nestin and also the presence of SP within the whole cell population of breast milk. They suggested that human breast milk may be a novel source of putative stem/progenitor cells with the presence of CD133 [4]. They reported the presence of other markers such as osteonectin, alkaline phosphatase and other neuro-epithelial lineage markers. Breast milk was also demonstrated to possess a sub-population of cells that show positivity for the markers of pluripotency such as OCT4, SOX2 and NANOG with their multipotent differentiation ability [6]. These findings suggested the existence of pluripotency in breast milk. Until then, only few markers had been found in breast milk. Later, many investigators worked on identification of integral cellular components of stem/progenitor cells of breast milk and more differentiated cells [7, 19, 20].

With the growing interest, we carried out a flow cytometric characterization of various stem/progenitor markers such as HSCs (CD34, CD133, CD117), MSCs (CD90, CD105, CD73), myoepithelial cells (CD29, CD44), immune cells (CD209, CD86, CD83, CD14, CD13, HLADR, CD45), as well as cell adhesion molecules (CD31, CD54, CD166, CD106, CD49d), SP (ABCG2) and CD140b in fresh samples of colostrum [5]. Although all these markers were present, their percentage varied from low expression to high to noteworthy. The presence of an array of various adhesion molecules in different proportions suggests the homing ability of these cells. They are responsible for proper growth necessary for neonatal development and homeostasis. It is predicted that a high expression of CD44 is required for firm adhesion of cell to endothelium. Besides, CD13 has also been identified as a potent marker that plays a vital role in angiogenesis and migration, apart from its immune activity [23]. Similarly, each cell adhesion molecule has its role in development and homeostasis in different mechanisms. More studies on cell adhesion molecules and their role might provide a clue to further understanding the migratory potential of these breast milk derived cells for their regenerative applicability. Thus, cells of breast milk favor the physiological needs of the neonate, making them vital for long-term use.

We found that MSCs were scanty in the whole cell population, which emphasizes that MSCs are not inherently present in human milk as in the case of other populations, as demonstrated previously. The percentage was not consistent among all individuals and it varied from individual to individual. This indicates the heterogeneity in the expression pattern between individuals. This was also reported in our previous findings [5].

To know more about breast milk cell under culture conditions, we isolated and cultured the breast milk-derived cell population (Fig. 2). We analyzed these cultured cells with those of an uncultured cell population for various markers using flow cytometry and the results are tabulated (Tab. 1). Scanty expressions of MSCs were identified in the primary culture, with more of epithelial ones in origin. Although researchers have demonstrated that breast milk possesses markers of MSCs, STRO1, CD105, CD44 and others are not restricted to mesenchymal cells. This raises a further question as to whether breast milk really possesses inherent MSCs. We found that its expression slightly increased upon further passages, representing a MSC-like phenotype. Along with other researchers, we also assumed that breast milk cell cultures are more of epithelial origin, with a transition to a mesenchymal-like phenotype in subsequent passages. This transition to a more MSC-like phenotype at a later passage may be due to the epithelial-mesenchymal transition [24].

In this article, we emphasized the hypothesis that breast milk may lack inherent MSCs, but attain mesenchymal stem-like cells upon subsequent passages mediated by epithelial-mesenchymal transition (EMT), which is further supported by other literatures [3, 7, 25]. EMT has been studied well in mammary tissues, but not much in breast milk. If the hypothesis of EMT in breast milk cultures is proven, then breast milk may be a good source not only for regenerative applications as mentioned above, but also for tumor studies, as EMT is the major cause of cancerous conditions.

**Growth factors**

The presence of growth factors in breast milk and their function is known worldwide. Great attention has been devoted to their physiological roles in the growth, maturation, and maintenance of neonatal organ development [9, 10, 26]. Among them, Vascular Endothelial Growth Factor (VEGF),...
Thus, assessment of these growth factors in various levels of breast milk plays an important role. Researchers have demonstrated high levels of growth factors such as EGF, VEGF and HGF in human breast milk that favor neonates in several ways [26]. Siafakas et al. have also shown high concentrations of VEGF in human breast milk and its binding to specific receptors on the cells of the intestinal epithelium [35]. As mentioned above, the results of our assessment of the most important growth factors, VEGF and HGF, in various breast milk populations from colostrum to mature milk are as follows. We showed that human milk, especially colostrum, revealed significantly higher levels of VEGF and HGF (1,542 ± 119 pg/ml and 7,129 ± 273 pg/ml) than did cord serum (16.63 ± 0.77 pg/ml and 2,582 ± 108 pg/ml, respectively) [28]. Besides, our assessment of growth factors over the period of lactation revealed the values of VEGF (pg/ml): 1,542 ± 119 to 1,041 ± 112, HGF (pg/ml): 7,129 ± 274 to 6,049 ± 118, and IGF-1 (pg/ml): 3,970 ± 200 to 3,018 ± 121, from

Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF), and Insulin Growth Factor (IGF) are those most studied. Interestingly, the concentration of many growth factors is higher in breast milk than in maternal serum and plasma [27]. We found that these growth factors are higher in human milk than in cord blood and cord serum [28]. They play a role in surfactant secretion, maturation of organs, especially pulmonary, the gastrointestinal tract and overall neonatal maturity [29, 30]. VEGF regulates angiogenesis and vasculogenesis [31], while HGF stimulates growth, motility, and morphogenesis in epithelial cells and other diverse cell types [32]. IGF-1 has been demonstrated to influence the production of VEGF and regulate the development of blood vessels [33]. Besides, it is an important growth hormone that mediates the protein anabolic and linear growth, thus promoting the effect of pituitary GH [34]. In the early postnatal period, EGF from breast milk helps in developing intestinal mucosa for continuing the process of cell proliferation, differentiation and maturation.

Figure 2. Cultured breast milk-derived cells in vitro. A. Cells at early P0. B. Cells at early P2. C. Cells at late P0. D. Cells at late P2 at confluence.
colostrum to mature milk, respectively [36]. These growth factors are found higher in colostrum than in mature milk. The concentration of growth factor and cell count keeps decreasing from colostrum to mature milk, as the stage of milk increases. This correlates with the higher physiological needs of the neonate in the first week after birth.

In a recent study, the secretion of growth factors and cytokines by MSCs along with their immunomodulatory properties are well described [37, 38]. In lieu of this, we determined if breast milk cultured MSCs may be a source of these growth factors. Hence, we determined the secretion of breast milk from cultured MSCs and found that MSCs secrete the growth factors VEGF and HGF without influence of any other sources such as serum [39]. The cultured MSCs of breast milk without serum revealed significant secretions of the VEGF and HGF growth factors (8.55 ± 2.26 pg/ml and 230.8 ± 45.99 pg/ml) as compared with MSCs of breast milk with serum (21.31 ± 4.69 pg/ml and 2,404 ± 482 pg/ml, respectively). This shows that breast milk secretes these growth factors without the influence of serum.

miRNAs in breast milk: key findings

Since its discovery, remarkable advances in the understanding of microRNA (miRNA) biology have been made, including the dissection of miRNA biogenesis pathways, the identification of numerous miRNA targets, and the study of

<table>
<thead>
<tr>
<th>SR. No.</th>
<th>Antigen</th>
<th>Fresh colostrum cells</th>
<th>Cultured breast milk cells</th>
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<tbody>
<tr>
<td>1</td>
<td>CD34</td>
<td>13.7 ± 2.0</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>CD133</td>
<td>43.1 ± 2.4</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>CD117</td>
<td>23.2 ± 1.51</td>
<td>28 ± 2.3</td>
</tr>
</tbody>
</table>

**Hematopoietic stem/progenitor cells**

**Mesenchymal stem cells**

**Myoepithelial cells**

**Monocytes/macrophages/dendritic cells**

**Cell adhesion molecules**

**Side population**

**Growth factor**

Values are represented as mean ± SEM.
their biological functions in physiological and pathological conditions [40, 41]. According to miRBase version 21.0 (http://www.mirbase.org) released in June 2014, 2,558 mature miRNAs have been identified, while the number of human protein-coding genes that are considered to be targets of miRNAs is estimated to be approximately 20,000-25,000 [42]. Therefore, a single mature miRNA can bind and regulate multiple miRNAs [43]. Importantly, ongoing research is discovering new miRNAs on various cells and tissues. Dysregulation of miRNAs leads to several pathological complications and diseases. Hence, recent evidence supports their use as a diagnostic marker.

miRNAs have several immune-related functions such as regulation of T and B cell development [43, 44], release of inflammatory mediators [45], proliferation of neutrophils and monocytes [46], and differentiation of dendritic cells and macrophages [47]. miRNAs are also thought to be involved in hematopoiesis [48], cardiac muscle development [49], insulin secretion [50], and neurogenesis [51]. Recent accumulating evidence suggests that human milk (HM, breast milk) is highly enriched in miRNAs. Kosaka et al. identified immune related miRNAs in breast milk [52]. Zhou et al. also identified 602 miRNAs from 452 pre-miRNAs in human breast milk exosomes [53]. They conclude that miRNAs that are transferred from mother to infant may be a new immune-regulatory agent in breast milk for infants. Similar to their work, many researchers have identified various miRNAs in different fractions of breast milk, which has been well summarized by Alsaweed et al. [54]. Hence, identification of miRNAs can therefore be employed to identify cell fate development and determination, for their therapeutic value and as biomarkers in normal physiology and pathological conditions such as cancer.

Breast milk cells: ray of hope to premature infants

Prematurity is a major determinant of neonatal mortality and morbidity [55, 56]. Premature infants suffer from several long-term health complications such as cerebral palsy, sensory deficits, learning disabilities, respiratory illness, gastrointestinal disorders and other diseases [56, 57]. Breastfeeding benefits preterm infants from a nutritional, gastrointestinal, immunological, developmental, and psychological perspective. However, mothers of preterm infants encounter a variety of breastfeeding barriers that result in decreased breastfeeding. To increase the incidence and duration of breastfeeding, interventions to support and educate breastfeeding mothers on the nutritional values of breast milk have been implemented in various hospital and community settings [58, 59]. This is due to the fact that India has the highest number of preterm deliveries with a rate of preterm birth of 21%. Most of the premature babies suffer from many health problems when compared to mature babies [27, 60].

The degree of prematurity may play a vital role in milk composition [8, 26, 61]. The preterm milk composition is directly correlated with the optimal nutritional and developmental status of premature babies who derive benefits from enhanced host defenses, neurological development, and gastrointestinal function. Besides, studies in preterm infants have shown improved feeding tolerance, lower infection risks and a decreased rate of necrotizing enterocolitis with an exclusive human milk diet [62]. Studies have also demonstrated differences between the macronutrient composition in term and preterm milk [63, 64]. However, the cellular constituents and benefits of preterm-delivered mother’s colostrum and mature milk are scantily explored.

Our study was a pioneer in the identification of the spectrum of cellular constituents of preterm colostrum and mature milk over the period of lactation. The components identified include epithelial/mammary stem cell population, hematopoietic/mesenchymal stem/progenitor cells, cell adhesion molecules and pluripotent markers that probably favor immunity, growth and cell fate development of premature infants (Tab. 2). The marker expressions were categorized and represented according to the previous publication [65]. Mature milk was found to represent 90% of water and 10% of nutritional compounds. However, it is well understood that not only colostrum but even mature milk possesses various integral cell components as identified by several researchers. This would enlighten the significance of preterm milk and strengthen the breastfeeding policy of preemies. A recent study on leukocyte populations in breast milk of mothers of preterm and term infants using multicolor flow cytometry concluded that fresh preterm breast milk is not deficient in leukocytes, but a minor difference might occur owing to the shorter gestational period [66].
From the aforementioned aspects, we report that breastfeeding would be the ideal medicine for premature infants even up to later periods of lactation. However, breastfeeding of preterm infants encounters many barriers. Hence, breast milk banks have emerged as a source of breast milk for neonates who cannot receive their own mother’s milk.

### Breast milk banking and need for breast milk cell banking: a new approach

Breast milk banking, as the name suggests, is the banking of human milk for the need of infants at the right time, when sufficient milk is not being produced by the mother herself. It is noted that more than 60% of the deliveries taking place in India are accompanied by several risk factors. Many premature babies are not receiving mother’s milk in sufficient amounts. The worldwide initiative to reduce infant mortality by promoting human milk feeding has been developed to reduce infant death, malnutrition and chronic illness, particularly in developing countries such as India, Sri Lanka, Bangladesh and several others in the Southeast Asian region where health resources are scanty. The World Health Organization and the American Academy of Pediatrics among many other health care organizations and associations agree that when a mother’s own milk is not available for fragile babies, the next best alternative is the use of pasteurized human milk, especially for the premature and high-risk infant population [67].

### Table 2. Antigenic expression of various markers of cells derived from preterm colostrum and mature milk.

<table>
<thead>
<tr>
<th>SR. No.</th>
<th>Antigen</th>
<th>Preterm colostrum</th>
<th>Preterm mature milk</th>
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<tbody>
<tr>
<td>Hematopoietic stem/progenitor cells</td>
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</tr>
<tr>
<td>1</td>
<td>CD34</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>CD133</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>3</td>
<td>CD117</td>
<td>L</td>
<td>M</td>
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<td>Mesenchymal stem cells</td>
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<tr>
<td>4</td>
<td>CD90</td>
<td>S</td>
<td>L</td>
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<td>5</td>
<td>CD105</td>
<td>L</td>
<td>H</td>
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<tr>
<td>6</td>
<td>CD73</td>
<td>S</td>
<td>S</td>
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<td>Myoepithelial cells</td>
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<tr>
<td>7</td>
<td>CD29</td>
<td>H</td>
<td>H</td>
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<td>8</td>
<td>CD44</td>
<td>M</td>
<td>L</td>
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<tr>
<td>Monocytes/macrophages/dendritic cells</td>
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<tr>
<td>9</td>
<td>CD86</td>
<td>M</td>
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<td>10</td>
<td>CD209</td>
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<td>11</td>
<td>CD83</td>
<td>S</td>
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<td>12</td>
<td>CD14</td>
<td>M</td>
<td>R</td>
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<tr>
<td>13</td>
<td>CD13</td>
<td>M</td>
<td>H</td>
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<tr>
<td>14</td>
<td>HLA-DR</td>
<td>M</td>
<td>H</td>
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<tr>
<td>15</td>
<td>CD45</td>
<td>H</td>
<td>L</td>
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<tr>
<td>Cell adhesion molecules</td>
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<tr>
<td>16</td>
<td>CD31</td>
<td>L</td>
<td>S</td>
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<td>17</td>
<td>CD54</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>18</td>
<td>CD166</td>
<td>S</td>
<td>L</td>
</tr>
<tr>
<td>19</td>
<td>CD106</td>
<td>S</td>
<td>S</td>
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<tr>
<td>20</td>
<td>CD49d</td>
<td>L</td>
<td>L</td>
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<td>Side population</td>
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<tr>
<td>21</td>
<td>ABCG2</td>
<td>S</td>
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<tr>
<td>Growth factor</td>
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<tr>
<td>22</td>
<td>140b</td>
<td>M</td>
<td>H</td>
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S: Sparse; L: Low; M: Moderate; H: High; R: Remarkable.
This has led to innovative clinical practices in neonatal intensive care units utilizing maternal and donor human milk. There are many breast milk banks all over the world, including India. India has a total of 17 milk banks, some of which are in Mumbai, Kolkata, Gujarat, Rajasthan, etc. The first mother’s milk bank in Asia was started in Mumbai on November 27, 1989. Mother’s milk banking has been increasing in the last few years. Breast milk banking ensures that the donor mother’s milk is safe, healthy and well-nourished, with no evidence of any disease conditions. According to WHO and UNICEF, globally only 20% of working women are able to breastfeed their children. Breast milk banking thus serves as a boon for working mother’s infants. Owing to the difference in composition of preterm and term milk, most milk banks separate “preemie milk” from “term milk”. Donor selection criteria include: healthy and well-nourished women, no evidence of tuberculosis/HIV/hepatitis or any other infection, not undergoing any hormonal treatment and not having any medications, and, most important, willingness to donate milk. The objectives of these milk banks are: 1. to ensure that every baby born in hospitals receive mother’s milk; 2. to avoid formula milk; 3. to increase the awareness and significance of breastfeeding; 4. most importantly, to lower the overall mortality rate and avoid malnutrition in countries. Those who would be benefitted by these banks are: VLBW babies especially in the first few days, lower segment Caesarean section (LSCS) deliveries, especially if LBW, multiple pregnancies, babies of mothers with problems, mothers who are not in a position to feed, babies with some diseases such as necrotizing enterocolitis and so on.

Recently, several integral cellular components and their growth factor secretions that would greatly favor neonates as discussed above have been identified in breast milk. With this advancement in research and identification of new cellular constituents in human milk, a major question that needs to be addressed is if these cellular constituents and growth factors will be preserved in pasteurized human milk banks. Will the cell components of breast milk be viable after pasteurization? Will breast milk be viable after long term preservation? Although human milk banking is quite beneficial to neonates, the nutritional components and cell components of this milk are equally important to be preserved for growth, cell fate determination and regenerative development. Thus, assessment of growth factors and integral cellular components and the viability of pasteurized and cryopreserved human milk becomes essential.

We postulate that cell components might not be preserved owing to the effect of pasteurization. Thus, infants might not receive the fullest potential of these cell components as mentioned above. Hence, we believe that heterogeneous cells from breast milk should be preserved like those of cord blood banking, thereby utilizing the fullest potential of donor milk for neonates. These cells, along with their secreted growth factors, may be a valuable source of regenerative therapeutics. These cells might also be used as an oral medicine for neonates after further investigations in the near future [68].

Conclusion

In summary, this article reviews the cellular components of breast milk, emphasizing its major stem cell constituents, derived growth factors and beneficial effects. We also emphasize breast milk banking and the need for breast milk cell banking for neonates. The cells derived from breast milk could be cryopreserved for use as a natural, oral medicine for the treatment of neonatal disorders after further investigations. This is also supported by another study carried out by Twigger and coworkers on how breast milk cells could be of use in central nervous system disorders [69]. Further intense research on identification of mammary stem cells/epithelial population/stem and progenitor population in breast milk and its marker characterization, coupled with miRNAs in breast milk, will serve many purposes: 1. for use in identifying the cellular origin of breast cancer and its therapeutic approach; 2. to identify the variations of this stem/progenitor population among various lactating preterm and term women, thereby acting as an indicator of abnormalities associated with low breast milk supply of various categories of women. This is in line with our previously published paper [5], where we proved that heterogeneity in breast milk cells varies in different lactating women; 3. to identify the key markers that are responsible for lactation and more milk supply and other key functions.

Overall, further research on breast milk and its cell components will pave the way to implementing the breastfeeding policy for better maternal and child health.

Declaration of interest

The Authors declare that there is no conflict of interest.
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