Comparative Analysis of SurCord® and CoreCyte®

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Summary

This review compares cellular and biochemical characteristics of cryopreserved human allografts, SurCord® (Surgenex®) and CoreCyte® (Predictive Biotech®). SurCord® is a minimally manipulated cryopreserved human umbilical cord allograft derived from donated C-section placenta. SurCord® is produced by using our proprietary process that allows preservation of native tissue architecture and retention of its biological activity. SurCord® contains a combination of viable cells, extracellular matrix and a wide array of regenerative cytokines and growth factors. According to the manufacturer's brochure, CoreCyte® is a minimally manipulated human tissue allograft derived from the Wharton's Jelly of the umbilical cord. CoreCyte® is processed to conserve the structural integrity of Wharton Jelly for homologous use and is cryogenically preserved. Based on the analysis, it was determined that the SurCord® product has superior characteristics in comparison with CoreCyte®, due to the following:

- **Cell count and viability:** After cryopreservation, SurCord® contained (1.83 x 10^6 of viable cells per mL), which is more than five times higher than CoreCyte® (0.37 x 10^6 of viable cells per mL).
- **Mesenchymal stem cell count:** Percentage of cells expressing mesenchymal stem cell (MSC) markers is similar in SurCord® and CoreCyte®. However, since the number of viable cells is much higher in SurCord®, the total number of viable mesenchymal stem cells is more than five times higher in SurCord® compared to CoreCyte® (415,410 vs. 85,100 of viable MSCs per mL respectively).
- **Hyaluronic acid content:** The concentration of hyaluronic acid per mL of SurCord® surpasses CoreCyte® by nearly twenty times (15,220 ng/mL vs. 777 ng/mL respectively).
- **Collagen content:** The amount of total collagen is 45 times higher in SurCord® compared to CoreCyte® (415 µg/mL vs. 9.2 µg/mL respectively).
- **No blood contamination:** Extracellular hemoglobin (red blood cell protein) is undetectable in SurCord®, while CoreCyte® contains 95 µg/mL of extracellular hemoglobin indicating blood contamination.

Method:

Both CoreCyte® and SurCord® are cryopreserved products. CoreCyte® arrived frozen on dry ice. SurCord® was stored in liquid nitrogen (-196°C) and was transferred to -80°C freezer the night before the experiment. For cell viability and MSC marker expression studies, both CoreCyte® and SurCord® were thawed and tested immediately upon arrival of CoreCyte®. According to CoreCyte® storage instructions, it can be kept frozen on dry ice or -80°C for 72 hours. Thus, for biochemical studies (HA and extracellular hemoglobin measurements), one vial of each CoreCyte® and SurCord® were retained in -80°C freezer and processed within 72 hours. Both allografts were processed in parallel using the same reagents.

Results

1.1 Visual appearance:

A difference in product color was discovered upon visual inspection. SurCord® has an opaque white color, while CoreCyte® has a distinctive reddish/brownish tint that may indicate the presence of red blood cells (Figure 1).

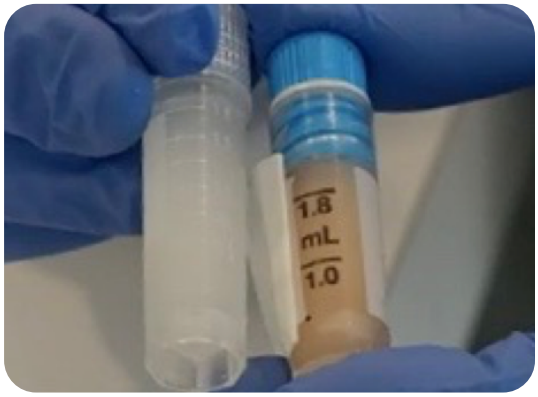

FIGURE 1. *Initial visual inspection showed the differences in product color. SurCord® (tube on the left) has an opaque white color, while CoreCyte®* (tube on the right) has a distinctive reddish/brownish tint that may indicate the presence of red blood cells.

1.2 Blood contamination:

The reddish tint of CoreCyte® may indicate the presence of blood. To probe for potential blood contamination, concentration of extracellular hemoglobin (red blood cell protein) in CoreCyte® and SurCord® was measured. Extracellular hemoglobin concentration was determined by measuring absorbance values at 578, 562 and 598 nanometers and applying the Kahn method for extracellular hemoglobin calculation [1]. Extracellular hemoglobin was undetectable in all tested samples of SurCord®, while CoreCyte® contained 95 µg/mL of extracellular hemoglobin indicating blood contamination. Extracellular hemoglobin is also an indicator of hemolysis (red blood cell lysis) and can cause system toxicity via multiple mechanisms, including cellular injury due to oxidation reaction, immune reaction amplification and others [2]. Moreover, red blood cell contamination may impair the efficacy of cell therapy [3].

1.3 Cell count and viability:

After cryopreservation cell count and viability are key characteristics in the evaluation of viable tissue allografts. Therefore, these parameters in SurCord® and CoreCyte® were compared. Cells were labeled with Acridine Orange/Propidium Iodode fluorescent stain (Logos Biosystems®). The number of total cells and cell viability were measured using a LunaStem® Automated Fluorescence Cell Counter (Logos Biosystems®) according to the manufacturer's instructions. The results are presented in **Table 1**. Tested vials of CoreCyte® contained a total of 1.4 x 10^6 cells per mL, however, on average, only 25% or 0.37 x 10^6 of these cells per mL were viable. In contrast, the cell viability of SurCord® is 51.6% or 1.83 x 10^6 viable cells per mL, which is more than five times higher than the number of viable cells in CoreCyte®. Finally, SurCord® demonstrated a higher level of consistency between lots, ranging from 41.1% to 63.8% of viable cells, while cell viability in CoreCyte® samples ranged from 10.9% to 38.3%.

Table 1. Viability and number of cells in SurCord® and CoreCyte®. All data are presented as Mean ± SD.

Product	Total cell count per mL, x 10^6	Viable Cells,%	Viable cell count per mL, x 10^6	Average size of nucleated cells, µm
SurCord®	3.96 ± 1.20	51.6% ± 9.8	1.83 ± 0.90	8.7 ± 1.0
CoreCyte®	1.42 ± 0.25	25.0% ± 19.0	0.37 ± 0.30	5.3 ± 0.21

1.4 Hyaluronic Acid Content:

Hyaluronic acid (HA) is a main component of articular cartilage and synovial fluid, which provides the backbone of large proteoglycan complexes. HA is viscoelastic and provides joint lubrication. In addition, HA creates an optimal 3D environment for cells and stimulates migration of synovial cells, chondrocytes and mesenchymal stem cells to the site of tissue defect [4]. As osteoarthritis (OA) progresses, HA concentration declines, which deteriorates viscoelastic properties of synovial fluid. The Hyaluronic Acid (HA) concentration was measured in SurCord® and CoreCyte® using Hyaluronan Quantitative ELISA Kit (R&D Systems®). Absorbance was measured using Synergy HT microplate reader (BioTek®). Data was analyzed using Gen5 software (BioTek®). The results demonstrated that HA concentration of SurCord® is almost 20 times higher when compared to CoreCyte® (**Table 2**).

Table 2. HA Concentration.

Product	HA Concentration, ng/mL
SurCord®	15,220
CoreCyte®	777

1.5 Collagen Content:

Collagen is a main component of extracellular matrix and a dominant structural protein of articular cartilage that serves to provide mechanical strength and to absorb shock. In osteoarthritis (OA), collagen network is degraded leading to progressive destruction of joints and the loss of function. Total collagen concentration was measured in SurCord® and CoreCyte® using Sirus Red Total Collagen Detection Kit (Chondrex®). Solid components of SurCord® and CoreCyte® were pelleted by centrifugation and solubilized in 0.05M Acetic Acid solution. Absorbance was measured using Synergy HT microplate reader (BioTek®). Data was analyzed using Gen5 software (BioTek®) (**Table 3**).

Table 3. Collagen Concentration.

Product	Collagen Concentration, µg/mL
SurCord®	415.0
CoreCyte®	9.17

1.6 Expression of mesenchymal stem cell markers:

The effectiveness of cord-based allografts against osteoarthritis is in part due to the presence of mesenchymal stem cells (MSCs). MSCs are capable of differentiating into various cell lineages, including chondrocytes, and of releasing a vast number of anti-inflammatory cytokines and tissue stimulating growth factors. There is no single marker for the identification of MSCs, but a combination of surface positive phenotypes such as CD90, CD73 and CD105, and negative blood markers including CD45, CD34, CD19, CD14 and HLA-DR is sufficient for MSCs characterization [5].

In order to determine the expression level of MSCs, SurCord® and CoreCyte® were treated with 0.1% Collagenase Type II solution to digest extracellular matrix and release cells. Digested mixtures were filtered to remove cell clumps. Cells were labeled with antibodies against mesenchymal stem cell markers including CD90, CD73 and CD105 (BioLegend®). Negative staining cocktail included antibodies against hematopoietic markers, such as CD45, CD34, CD19, CD14 and HLA-DR (BioLegend®). Corresponding isotype controls were used to monitor the level of background fluorescence. Fluorescent signals were detected by FACSVerse flow cytometer (BD Biosciences®) and data were analyzed by FACSuite (BD Biosciences®) and FlowJo (FlowJo LLC®) softwares. In addition to cells expressing only one of the MSC markers, the percentage of cells concurrently expressing all three MSC markers (CD90, CD105, and CD105) was calculated. The results are presented in **Figures 2, 3** and **Table 4**.

Our data showed that the percentage of cells expressing CD90, CD105 and CD73 surface markers are similar in SurCord® and CoreCyte®. However, since the viable cell count is much higher in SurCord®, the total number of viable mesenchymal stem cells is also significantly higher in SurCord® compared to CoreCyte® (**Figure 3**).

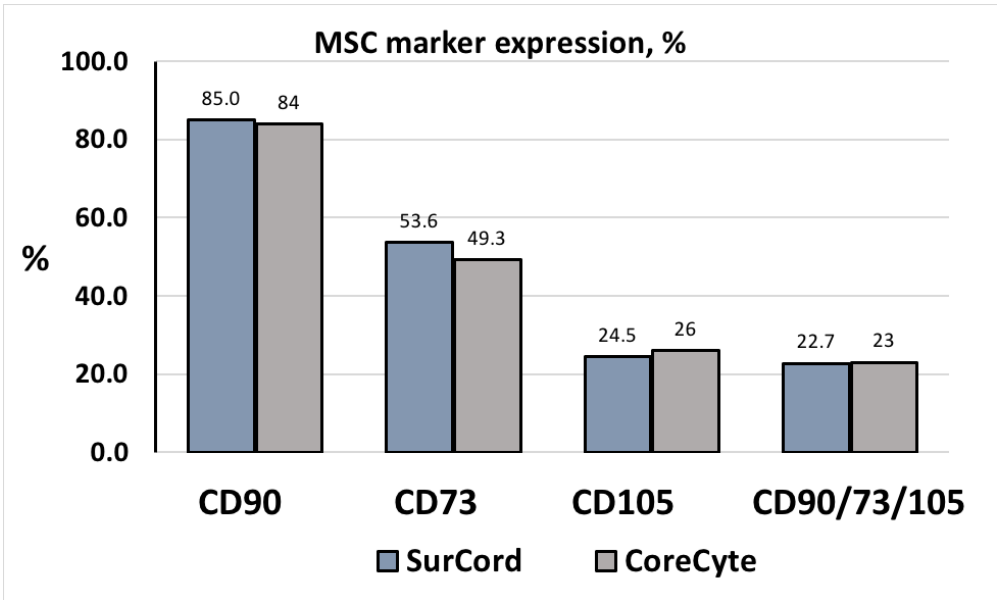

FIGURE 2. Mesenchymal stem cell marker expression in SurCord® and CoreCyte®

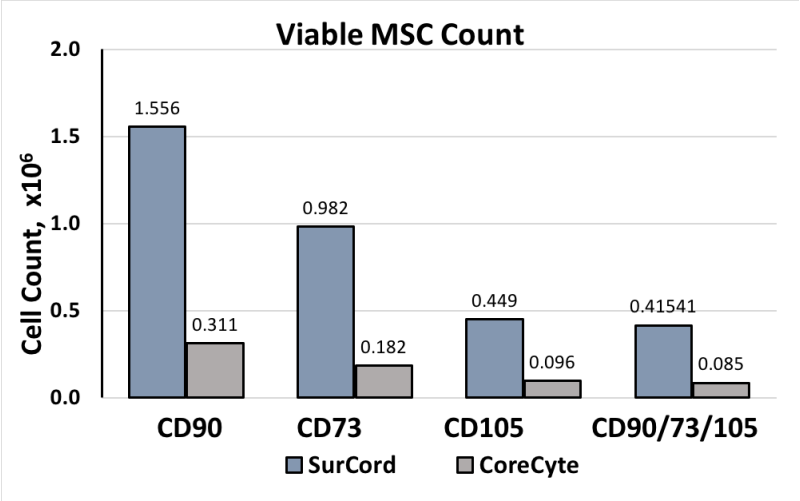

FIGURE 3. *Mesenchymal stem cell marker expression in SurCord® and CoreCyte®.*

Table 4. *Mesenchymal stem cell marker expression in SurCord® and CoreCyte®.*

Marker	SurCord®	SurCord® viable cell count, x 10^6	CoreCyte®	CoreCyte® viable cell count, x 10^6
CD90	85%	1.556	84.0%	0.311
CD73	53.6%	0.982	49.3%	0.182
CD105	24.5%	0.449	26.0%	0.096
Concurrent expression of CD90/CD73/CD105	22.7%	0.415	23.0%	0.085

Conclusion

In review, testing showed SurCord® contained higher levels of MSCs in comparison to CoreCyte®. Cell viability and MSC count testing showed that SurCord® contained 415,410 viable cells expressing all three MSC markers while CoreCyte® contained only 85,100 viable cells expressing all three MSC markers. ELISA testing showed that SurCord® contained nearly 20 times the HA content and 45 times more collagen than CoreCyte®. In addition, CoreCyte® showed signs of blood contamination containing 95 µg/mL of extracellular hemoglobin while SurCord® extracellular hemoglobin amounts were undetectable.

References

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