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# Proteomic Profiling of Human Umbilical Cord Blood Serum and its Potential Use in Regenerative Therapies

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**Abstract:** Application of mediators directly to the injured site to promote de novo tissue can be the most promising strategy in regenerative medicine. However, understanding the mechanism of regional application of proteins /growth factors is challenging. Likewise, the proteins from Human Umbilical Cord Blood Serum(hUCBS) remain inadequately understood in terms of its therapeutic use. Several biological processes of the components in hUCBS remains unanswered. Human umbilical cord blood was collected in vacutainer tubes from 35 healthy mothers.hUCBS was obtained after centrifuge -4$^{o}$C at 10,000 rpm for 20minutes. After the removal of high abundant proteins, proteomic profiling of UCBS was done by Mass Spectrometry. The Mascot search engine was used for peptide sequence identification. Proteins identified were assessed for tissue expression, cellular components, and biological functions based on Gene Ontology (GO) analyses. A total of 99 proteins were detected. The tissue expression information of proteins obtained included the Stomach, Liver, Heart, Lungs. Around 91 proteins were expressed in embryo organs. Proteins in hUBCS reflect the physiological status of the fetus/pregnancy. Major proteins found in hUCBS were expressed in the embryo, indicating that proteins were involved in organ development. Data contributed to a better understanding of the protein functions present in hUCBS.

**Keywords:** umbilical cord blood serum; proteome; regeneration; mass spectrometry.



## 1. Introduction

The principles of tissue engineering and regeneration involve conduction, induction, and cell transplantation [1]. The conduction of adjacent cells, vasculature available, defect size, the speed at which the cell migrate are all factors responsible for conduction and induction in regeneration to restore the existing small amount of tissues [2]. Bioactive factors emphasize inducing new tissues through the effect of recruitment, proliferation, and differentiation of cells; for example, Bone Morphogenic Protein (BMP) inducing new bone [3,4].

Use of hUCBS as a cell medium for isolation stem cells as an alternative to Fetal Bovine Serum(FBS) exhibited enhanced self-renewal of bone marrow-derived mesenchymal stem

cells and to differentiate into adipocytes and osteocytes. It was seen that human pluripotent stem cells maintained pluripotency, differentiation capacity, and karyotypic stability when the hUCBS-containing medium was used in culture. This proved that hUCBS was an efficient culture for the growth of stem cells, owing to its constituents [5,6].

The human umbilical cord, a placental tissue derivative, has been used for therapeutic purposes. The human umbilical cord used as dehydrated and processed (PURION® PLUS) form for the purpose of wound healing was revealed in a study for wound healing. About 461 protein biomolecules were detected in the dehydrated cord. The study showed the profound healing of chronic and acute wounds in rat models. The material also showed biodegradation in-vivo with excellent wound closure. These include factors such as Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-beta 1 (TGF-β1), angiogenin-4, and Platelet-Derived Growth Factor-AA (PDFG-AA) were detected. Inflammatory modulators, such as Interleukin-1 beta (IL-1β) and Monocyte Chemoattractant Protein-1 (MCP-1), proteases, and inhibitors, were also present. Adhesion molecules, such as Vascular Cell Adhesion Protein-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1), membrane-bound cell receptor proteins, and signaling receptors were identified in the same sample. Many signaling factors required for tissue regeneration were also present, thus making dehydrated umbilical cord tissue, most bioactive tissue [7]. When compared to human blood derivative, the use of umbilical cord and cord blood derivatives in therapy have shown efficient results. Also, a study showing hUCBS when applied topically on third-grade thermal burns in the rat model demonstrated accelerated healing with enhanced angiogenesis and anti-inflammatory effects [8].

Therapeutic use of hUCBS as eye drops after chemical injury resulted in significant corneal epithelization compared to other blood derivatives like human blood plasma serum. It was seen that studies with hUCBS eye drop on rat models for corneal epithelisation demonstrated faster epithelialization and profound anti-inflammatory reaction by downregulating expression levels of TNF-α and MMP-9 and decreasing the expression of IL-1β levels [9]. Studies on hUCBS eye drops demonstrate promising cure in human subjects and rat models for acute chemical burns, persistent epithelial defect, ocular graft-versus-host disease, recurrent corneal erosion, neurotrophic keratitis, dry eye disease, Hansen's disease, and laser epithelial keratomileusis. Not only did it prove to be effective, but it also resulted in favorable effects for long-term treatment[10]. Extensive use of hUCBS for the cure of ocular surface diseases has turned out to be appreciable in terms of competency and safety when compared with other autogenous and allogenic blood derivatives [11,12,13,14]. Cord blood serum-based therapy has shown to broaden its potential role in patients with glaucoma, proving to have a neuroprotective role on topical application suggestive of elevated growth factors in its composition [15].

Due to minimal clinical data on its topical use for ophthalmological disorders and skin regeneration, development and validation of its use need to be addressed. Allogenic hUCBS as therapeutic material needed more clinical testing and detailed analysis to address issues with respect to accurate function, biovariability among donors, optimal dosage, and formulation. An allogenic biotherapeutic material like hUCBS would require further clinical trials and elaborate analysis to address issues like variability among samples, functional accuracy, formulation, and ideal dosage.

In the present study, hUCBS proteins were identified using Mass spectrometry. A detailed analysis of the proteins present in hUCBS may provide a basis for its therapeutic use.

## 2. Materials and Methods

*2.1. Samples collection and preparation.*

The cord blood was collected from mothers identified prospectively, and informed written consent was obtained as per the Ethical Committee protocol. Screening for Syphilis, Hepatitis B, Hepatitis C, and HIV was done. Human umbilical cord blood was obtained from 35 healthy mothers soon after delivery. Pregnant women with a history of pre-existing hypertension, anemia or diabetes, premature or induced labor, drug use or tobacco, and also reporting of other medical or obstetric complications were excluded from the study.

Immediately after the childbirth, the umbilical cord was secured using 2 clamps. The cord blood was extracted without the use of anticoagulants using a sterile syringe (Figure 1a). The blood was then transferred into Gold top BD vacutainers coated with serum separator/ clot activator and was allowed to stand for 8 hours at a room temperature of 25ºC for clot formation and serum separation (Figure 1b). The straw-colored serum was collected and stored in red top vacutainer tubes until further use. After an estimated 60ml of pooled serum sample was obtained, the sample was thawed in a water bath at room temperature. The serum was then transferred into sterile 10ml Eppendorf and was subjected to centrifugation in a cooling centrifuge at-4ºC at 10000 rpm for 20 minutes. The pure serum was isolated and transferred into sterile Eppendorf (Figure 1c). Samples were processed for albumin and IgG removal using Proteoprep Blue Albumin & IgG Depletion Kit (Sigma Aldrich). 1 ml of thus obtained serum was subjected to proteomic analysis using Nano LC-MS/MS analysis using OrbitrapFusion™ Tribrid™ Mass Spectrometer. The parameter was set to enzyme trypsin digestion; fixed modification was set as Carbamidomethyl(C), variable modifications at Acetyl (Protein N-term), oxidation (M), Peptide mass tolerance at 10 ppm, fragment mass tolerance at 0.2 Da, maximum of 2 missed cleavages were allowed, False discovery rate was defined at1%. The Mascot algorithm (Matrix Science, Boston, MA, USA) was used to identify the peptide sequences present in the protein sequence database.

## 3. Results and Discussion

*3.1. Distinct Proteins detected in hUCBS.*

In this study, proteins of umbilical cord blood serum were s isolated and analyzed using Nano LC-MS/MS analysis. The hUCBS was enriched with 99 proteins after the depletion of high molecular weight proteins.

*3.2. Characterization of hUCBS proteome.*

3.2.1. Subcellular localization.

The proteins that were identified in this study were verified by UniprotKB and GO analysis and categorized according to their cellular localization. The most abundant proteins were derived from the followed by extracellular region proteins (64%), plasma membrane proteins (14%), unknown or unclassified proteins (10%), cytoplasm (5%), endoplasmic reticulum (4% ), and nuclear proteins (2%) and mitochondria (1%) (Figure 2).

3.2.2. Tissue expression.

The tissue expression was searched based on UniprotKB, Bgee version 14.1, and GO database. Genes expression was categorized into 22 tissue based on the number of hits per organ. The majority of the proteins were attributed to organs like stomach/intestine, spleen, liver, heart, testis/penis, embryo, and blood/plasma. Around 91 proteins identified were expressed precisely annotated in embryo tissue. When compared to proteome studies of amniotic fluid, it was seen that much lesser, about 24 proteins were expressed in the embryo. [16] (Figure 3).

3.2.3. Assignment of biological process.

By utilizing UniprotKB, and GO database functions of each protein was retrieved. About 16 functions were identified and matched with the protein function. Protein involved in major functions included adaptive immunological response, hematological response, transport, protease inhibitor, and lipid metabolism (Figure 4).

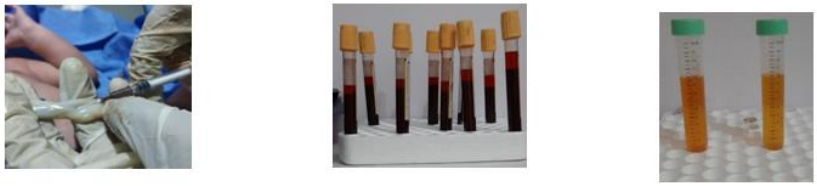
In spite of the significant use of hUCBS for various treatments, few studies mention extensive proteomic analysis in terms of quantitatively analyzing the constituents. Moreover, previous studies explain the proteome of hUCBS of pathological conditions and the identification of differentially expressed proteins in diseased conditions [17,18]. The entire molecular functions of the proteins in hUCBS are so far not reported. In the current study, an effort is made to quantitatively analyze the proteins attained in the sera preparation. The diverse aspects of 99 proteins obtained from hUCBS preparation, which includes subcellular functions and tissue expressions, were explored utilizing publically available databases. About 64% of the protein could be found in extracellular space; on the contrary, 4% protein in cord blood and 42% supernatant amniotic fluid proteins were localized extracellularly [18,16].

Proteins found in hUCBS reflected as particular metabolic function and factors in the growth of the fetus. Neonatal proteins are highly expressed in hUCBS; this finding draws a parallel connection to proteome studies done on neonatal hUCBS[17]. Some of these proteins that are uniquely found in the umbilical cord blood may be essential for fetal development. Proteins of hUCBS were also expressed in other gestational tissue like placental tissue and amniotic fluid. The majority of the proteins were also expressed in blood plasma. This indicates that predominant proteins detected are cellular in nature from the fetal or placental origin.

On characterization, significant functions of hUCBS were adaptive immunity, immune response, hematological system development, transport system, and protease inhibitor. hUCBS was obtained towards the completion of pregnancy, and most of the proteins could have been directly obtained from mothers' circulation featuring predominantly adaptive immunity and immune response functions [17].

Insulin-like growth factor-binding protein 2 was identified in the sample, indicates a significant role in regeneration. Due to variable factors influencing the cord blood sample collection, a vast discrepancy was seen with the growth factor reported [17,18]. To overcome the limitations of variable factors, multiple health donors and formulation of the serum containing desirable growth factors and functions have been suggested [19,20]. Studies have also suggested the collection of cord blood from young mothers and prolonged duration of labor could increase the desired growth factors like epithelial growth factor [21]. It is possible that the presence of proteins in the noncellular supernatant of cord blood serum is closely

associated with molecular and physiological pathways operation during pregnancy. Presence of proteins in hUCBS were predominantly recognized in embryo organ. Consequently, protein functions could be more closely linked to organ development.



**a** **b** **c**

**Figure 1.** Procedure for preparing hUCBS: (a) collection umbilical cord blood ; (b) cord blood collection in vacutainer tube ;(c) pooled umbilical cord blood serum collected after centrifuge.

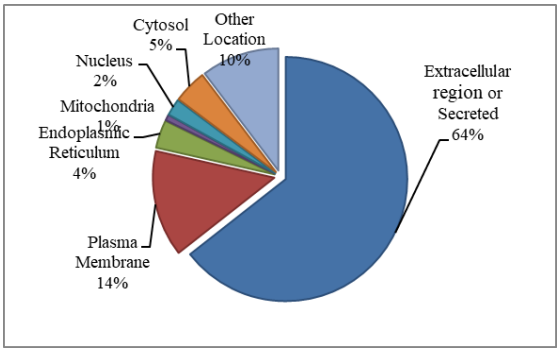



**Figure 2**. Identified proteins in hUCBS are classified by subcellular location.

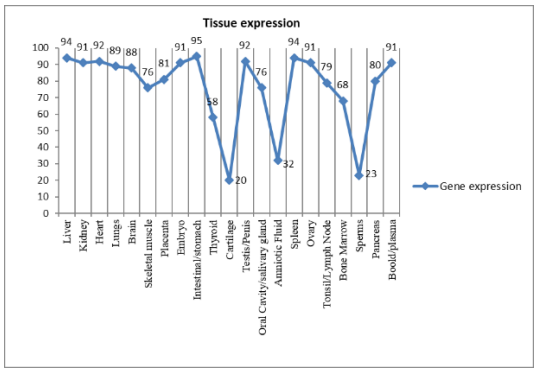



**Figure 3.** Tissue expression profile of hUCBS proteins expressed in major tissues in humans.

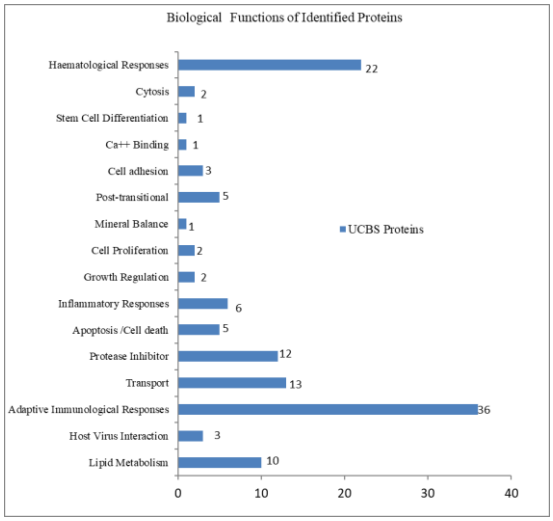



**Figure 4**. The Gene Ontology enrichment analysis for biological functions of the proteins identified from hUCBS matched the number of associated genes that are identified for each function.

## 4. Conclusions

It can be noted that hUCBS is a dynamic complex mixture. Various characteristic of hUCBS was surveyed, including functions and tissue expression. Data of the present study contribute to a better understanding of hUCBS functions and may provide the basis for further compositional and functional studies.

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## Conflicts of Interest

The authors declare no conflict of interest.

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