

Interaction of Citrate-Stabilized Gold Nanoparticles with Umbilical Cord RBC

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Abstract

Abstract: Umbilical cord blood contains stem cells which are useful in chemotherapy, implantation; however, cord RBC is considered as a waste product. It is necessary to use the umbilical cord blood for neonatal blood transfusion to avoid diseases. Nowadays, nanoparticles are used in biomedical and pharmaceutical industry. Nanoparticles interact with blood via different routes. In addition, these nanoparticles used in biomedicine, drug delivery, bioimaging, and to increase the shelf life of RBC. Nevertheless, research of nanoparticle interaction with umbilical cord red blood cells is still insufficient. Thus, in the present study, interaction between umbilical cord blood-RBC and citrate-stabilized gold nanoparticles is studied.

Methods: Umbilical cord rbc isolated from whole blood immediately after the collection using centrifuge. PBS buffer is used as a physiological media and washing reagent. After, interaction of isolated RBC (2% suspension) with different concentration of citrate stabilized gold nanoparticle were investigated using UV-Visible spectroscopy, Raman spectroscopy, and Interfacial tension measurement technique.

Results: UV-Visible spectroscopy is used to investigate the changes in Soret band and oxygenation bands (Q1 and Q2). Variation in absorption intensity is observed in umbilical cord RBC incubated citrate stabilized gold nanoparticles. However, there is no significant shift in Soret band and Q1, Q2 band of cord-RBC with Au nanoparticles. In case of Raman spectroscopy, enhancement in disulphide band region (526 cm⁻¹ and 579 cm⁻¹) and change in the spin state marker is investigated. While, interfacial tension decreases for 10 µl and increases for 100 µl and 200µl of 40 nM AuNP.

Conclusion: The umbilical cord rbc remains unaffected by 10 µl of citrate-stabilized gold nanoparticles (40 nM).

Keywords: Umbilical cord RBC, citrate stabilized gold nanoparticles, UV-visible spectroscopy, Raman, IFT

Introduction

Umbilical cord blood RBC differs from adult RBC in many aspects and has a greater affinity for oxygen. Cord RBC shows distinguishing characteristics such as cell membrane composition, morphological structure, surface tension, viscosity, pH, haemoglobin concentration, and oxygen binding affinity [1, 2]. Generally, cord RBCs are considered as a waste product but to some extent they can be used for blood transfusion in neonates [3]. Adult RBC transfusion in the neonate may lead to various diseases related to respiratory, lung, and eye. Therefore, it is necessary to store cord RBCs for a long duration due to their unique features and small volume availability. Umbilical cord blood contains stem cells which can produce other types of cells. These cells are used for transplantation in cancer therapy [4]. Cord RBCs have a short life span of 45-70 days as compared to adult RBCs of 120 days. The large size of cord RBC with more cell rigidity makes the cell less deformable within the small blood vessels. Cord blood-RBC contains more lipids, phosphorous, and cholesterol per cell than adults [5]. Cord RBC shows a decrease in sedimentation rate and rouleux formation. Osmotic fragility of cord RBC is lower as compared with adult, due to pH values. The concentration of Haemoglobin in the cord (15.7-17.9 g/dm³) is significantly higher than that of Adults [6],[7]. Cord blood contains different Haemoglobin i.e. HbA, HbF, and HbA2. Fetal haemoglobin (HbF) has a higher affinity to bind to oxygen than adults. Cord RBC membrane becomes less dielectric as compared with adult RBC when measured by the dielectrophoresis technique. Cord blood has high-level proteins, K, Ca, P, and folic acid. Cord blood contains hematopoietic stem cells (HSC) which make every type of cell in the blood. Transplants of HSC from cord blood are used to treat blood diseases like leukemia. RBCs from cord blood contain fetal Hb which is dominant in newborns, therefore cord RBCs are more suitable for neonatal transfusion. Cord RBC is less deformable than adult RBC due to lower elastic compressibility, structural, and permeability differences. Cord RBCs are more prone to mechanical stress than adult cells [11]. Cord RBCs have a high percentage of cholesterol and phospholipids with lower membrane fluidity. Lipid composition is important in part of the physical properties of cord-RBC membranes [12]. Cord-RBC is major parameter to understand the health status of newborn baby and mother [15]. During pregnancy, health of the mother plays important role in blood rheology of baby. Pathophysiological investigation of Cord RBC like RBC aggregation, sedimentation rate provides information whether the mother suffered from hypoxia, hypertension, thalassemia, etc. Low viscosity of plasma and less proteins leads to RBC aggregation in cord blood [16], [17]. Cord RBC contains more membrane-bound Haemoglobin than adults. Fetal Haemoglobin is more susceptible to oxidation stress than HbA and protects proteins against the stress [18]. Iron oxide nanoparticles (SPION) and cord blood-derived natural killer cells are used for cancer cell therapy [3]. Targeted transplantation of cord blood with immunomagnetic nanoparticles was used to repair corneal defects [8]. Stem cells present in the umbilical cord RBC are used in the defense mechanism of lymphoma, and leukemia. Recently, it has been reported that damaged stem cells due to certain medical reasons can be repaired using nanoparticles and drugs [9]. Some nanoparticles like silica-coated magnetic nanoparticles are used to label images. Ektacytometry, UV-visible spectroscopy, Blood Gas analysis, FTIR, Mossbauer spectroscopy, Raman spectroscopy, Dynamic Light Scattering, AFM, SNOM Micropipette aspiration techniques are used to understand the biophysical state of RBC. Very few reports are available on umbilical cord RBC-nanoparticle interactions. Despite the difference in physiology (specifically with regard to umbilical cord red blood cell) between umbilical cord red blood cell (UCRBC) and adult red blood cell (RBC), few studies to date have focused on how nanoparticles interact specifically with UCRBCs. Citrate stabilized gold nanoparticles were used in labelling, immobilization of protein, biosensing, and targeted drug delivery application. This article provides new information in an area that has not been studied extensively; therefore, contributing additional information to the overall body of knowledge regarding neonatal transfusion and the use of nanomedicine. In the present work, umbilical cord RBC-citrate stabilized gold nanoparticle interactions are investigated, UV-visible spectroscopy, Raman spectroscopy, and Interfacial tension measurement techniques.

Materials and Methods

Chemicals: Gold nanoparticles (5 nm) were procured from Sigma Aldrich; the solution is stabilized by using Citrate buffer with O.D. 1, phosphate buffer saline.

Cord blood Collection: Cord blood was immediately collected after delivery in a standard hospital under the supervision of a medical expert and stored in an EDTA vial. Ethical approval for this study was obtained from Savitribai Phule Pune University ethical committee with the consent of subject (Ref. No. SPPU/IEC/2021/129).

Cord blood was collected from standard hospital under the supervision of gynecologist and store in EDTA vial. Cord red blood cells were isolated from whole blood using centrifugation for 10 minutes at 25 °C at 3000 rpm. RBCs were washed with PBS buffer saline pH-7.34 for three times. For experiment purposes, 2% suspensions of Cord-RBC were prepared with PBS as a diluting reagent. All experiments performed with Cord-RBC were conducted in accordance with the rule of Institutional ethical committee. Ethical approval (Ref. No. SPPU/IEC/2021/129) for this study was obtained from Savitribai Phule Pune University ethical committee with subject consent.

Umbilical cord blood RBC-citrate stabilized gold nanoparticles

Cord RBCs were treated with citrate-stabilized gold nanoparticles with concentrations of 10 μ l and 100 μ l of 40 nM for 12 hr of incubation at 37 °C. Characterization was performed using salinity test, UV-visible spectroscopy, Raman Spectroscopy, and Interfacial tension measurement (IFT) techniques.

Results and Discussions

UV-visible spectroscopic study of citrate stabilized Au nanoparticle interaction with Cord blood-RBC

UV-visible spectra measurements were performed using 2% suspension of cord RBC and RBC incubated with citrate stabilized gold nanoparticles for different volumes i.e. 10 μ l, and 100 μ l of 40 nM in PBS buffer. Absorption of the haem group inside the RBC and its associated oxygenation bands i.e. Q1 and Q2 were examined in the present study. Absorption spectra of Cord RBC-Au nanoparticle interactions were recorded using a UV-visible spectrophotometer (JASCO - 760).

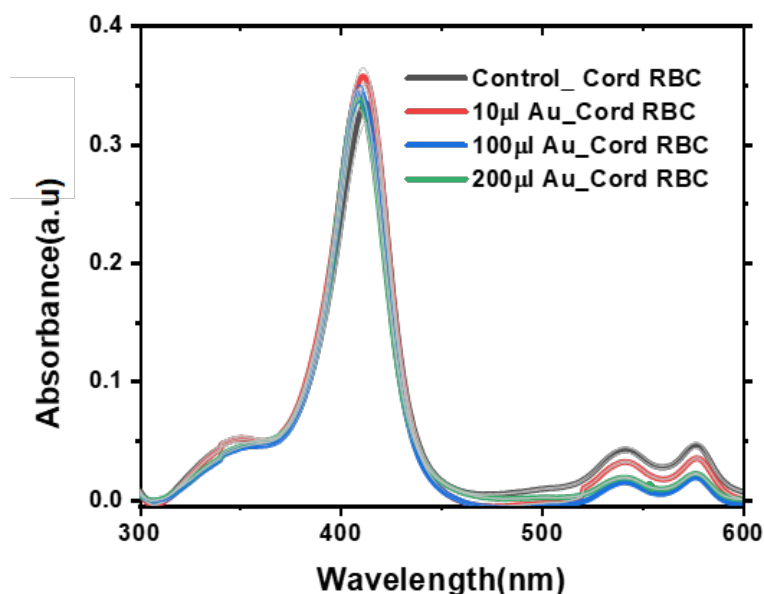


Figure 1: UV-visible spectroscopy of Au nanoparticles treated Cord RBC

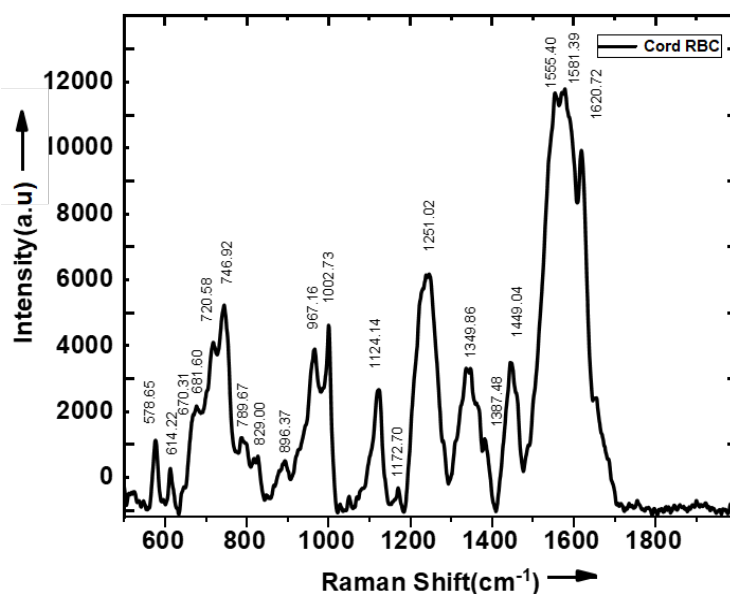
Table 1: Soret Band and Q Band Associated With Cord Blood -RBC Incubates With Different Concentration of Gold Nanoparticles

Band	Cord RBC	Cord RBC:	Cord RBC:
	Control	10 μ l AuNP(40nM)	100 μ l AuNP (40nM)
Soret Band	412 nm	411 nm	409 nm
Q1	543 nm	543 nm	541 nm
Q2	578 nm	578 nm	577 nm

The peaks at 281 nm, 347 nm, 420 nm (Soret band), 543 nm (Q1), and 578 nm (Q2) can be seen using UV-visible absorption spectroscopy of Adult RBC and Cord RBC, which is shown in Figure 1. The Soret band peak is located at 420 nm in the UV-visible spectra of umbilical cord RBC. Similarly, the oxygenation state is represented by the Q1 and Q2 peaks at 543 nm and 578 nm, respectively. Citrate-stabilized gold nanoparticles incubated in umbilical cord RBC reveal variation in absorption intensity (see Table 1). However, there is no significant shift in Soret band and Q1, Q2 band of cord-RBC with Au nanoparticles.

Raman spectroscopic study of Au nanoparticle interaction with Cord-RBC

Raman spectra of cord RBC and citrate-stabilized gold nanoparticles were recorded using a Renishaw spectrophotometer (inVia) which is shown in Figure 2. RBC was incubated with 40 nM concentration of Au nanoparticles for 4 hr. The laser power delivered to the sample was 15 mW to avoid photodamage. One drop of 15 μ l Cord - RBC and citrate stabilized Gold nanoparticle-incubated cord-RBC was drop cast on the Aluminum substrate and samples were settled for 15 min.

**Figure 2:** Raman Spectrum of Isolated Cord RBC from Whole Cord Blood

Raman peak and its signature associated with umbilical cord -RBC is as follows

Spin state marker region is in between 1500-1640 cm⁻¹ appearing at 1550 cm⁻¹, 1581 cm⁻¹, and 1620 cm⁻¹.

Pyrrole ring vibration modes of Haemoglobin are observed at 1387 cm⁻¹, 1371 cm⁻¹, 1349 cm⁻¹ which corresponds to half ring symmetric stretch.

Ring deformation is observed at 670 cm⁻¹ and 849 cm⁻¹ is assigned to single-mode stretching vibrations for the amino acids

and valine polysaccharides.

The peak position at 720 cm⁻¹, 968 cm⁻¹, 1738 cm⁻¹ corresponds to lipid assignment, 1125 cm⁻¹ is appearing for $\nu(\text{C}=\text{C})$ Acyl backbone in lipid.

The peak associated with 614 cm⁻¹ is for cholesterol ester, Nucleic acid is appearing at 829 cm⁻¹, 1340 cm⁻¹

Sulphide stretching is observed in protein at 526 cm⁻¹, phosphatidylserine and phosphodiester is appearing at 579 cm⁻¹ and 896 cm⁻¹, Cytosine, guanine and tyrosine assignment is at 1173 cm⁻¹ and 1248 cm⁻¹.

C-O stretching, C-N stretching of protein is observed at 1053 cm⁻¹, CH₃ band at 1384 cm⁻¹, C=O at 1717 cm⁻¹ and 1759 cm⁻¹ is assigned for C=O stretching.

The detail information related to band assignment is provided in Table 2.

Table 2: Umbilical Cord blood-RBC with Peak Assignment

Cord RBC Peak	Intensity	Assignment
526.4857	0.05395	$\nu(\text{S-S})$; S-S sulphide stretching in proteins
579.334	0.17407	Phosphatidylserine
614.5662	0.1066	Cholesterol ester
670.31	0.1	$\nu_7(\delta)$: porphyrin deformation)
720.2629	0.40472	Lipid assignment
747.4878	0.49089	--
829.1625	0.13652	$\nu_2 \text{ PO}_2^-$ stretch of nucleic acids
849.9815	0.04627	Single mode stretching vibrations for the amino acids and valine and polysaccharides, Tyrosine (Fermi resonance of ring fundamental and overtone)
896.424	0.1263	Phosphodiester
968.4899	0.38843	Lipids
1003.722	0.44101	Phenylalanine, C-C skeletal
1053.368	0.04271	C-O stretching, C-N stretching(protein)
1125.433	0.29357	$\nu(\text{C}-\text{C})$ Acyl backbone in lipid (Trans confirmation)
		C-N stretching vibration (Protein vibration)
		$\nu(\text{C}-\text{O}) + \nu(\text{C}-\text{C})$
1173.477	0.0614	Cytosine, guanine, Tyrosine (collagen type I)
1248.746	0.56462	Guanine, cytosine (NH ₂)
1340.03	0.34362	Nucleic acid modes
1384.871	0.1777	CH ₃ band
1448.929	0.35658	CH ₂ CH ₃ deformation, CH ₂ deformation, CH ₂
1557.829	0.98981	$\nu(\text{C}=\text{C})$, porphyrin, Tyrosine, amide II, COO ⁻ , Tryptophan, $\nu(\text{C}-\text{N})$ and $\delta(\text{NH})$ amide II (protein assignment)
1581.851	1	Pyrimidine Ring (Nucleic acid and heme protein)

1621.888	0.85543	v(C=C), porphyrin
1717.975	0.02558	C=O
1738.794	0.02645	lipids
1759.614	0.03217	C=O stretching

Raman spectra of Cord RBC incubated with Gold nanoparticles

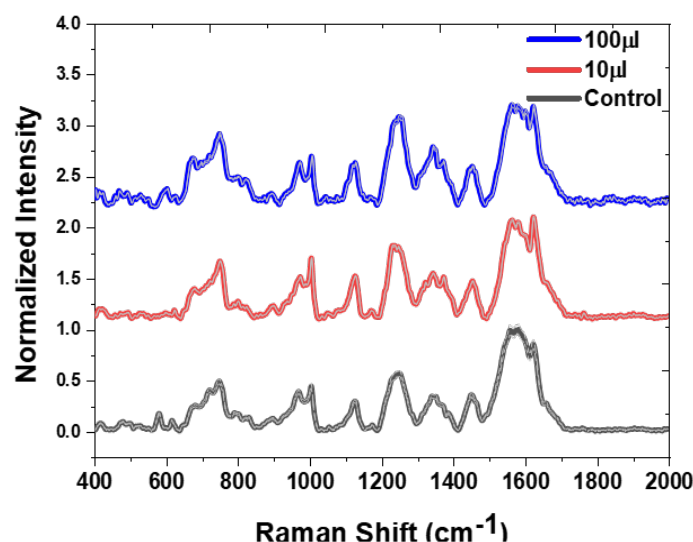


Figure 3: Raman Spectra of Cord RBC Incubated with Gold Nanoparticles with Different Concentrations a) control b) 5 μ l c) 10 μ l d) 100 μ l

The Raman peak position at 526 cm^{-1} and 579 cm^{-1} showed the presence of disulphide bridges. An increase in peak intensity at positions 526 cm^{-1} and 579 cm^{-1} suggests that cord RBC treated with gold nanoparticles modifies bridges in the disulfide region. These disulphide bridges are connected to the membrane's cysteine residue. Any alteration in secondary and tertiary structure of protein or disturbance in cellular environment affects the disulphide bond. An increase in disulphide-related peak intensity or a shift in peak position can reflect enhanced disulphide bond formation, often associated with oxidative stress or protein cross-linking. The disulphide bond is impacted by any change in the secondary and tertiary structures of proteins and also by disruptions in the cellular environment. Increased disulphide bond production, which is usually attributed to oxidative stress or protein cross-linking, could be indicated by a rise in disulphide-related peak intensity or a change in peak location. This result is consistent with bond formation between protein-glutathione and sulfide bridge. The changes in disulphide region is due to oxidative stress developed in RBC membrane and cytoskeleton proteins such as spectrin and band 3. According to literature, spectral changes observed in red blood cell is correlated with oxidative modification in RBC membrane proteins, which provide the information of biochemical studies of umbilical cord rbc [19,20]. In addition, Raman spectra also provide the information of spin state marker. The spin state marker (1500-1640 cm^{-1}) is an essential indicator of membrane fluidity or protein-lipid interaction. In the Figure 3, CH₃ band disappears at citrate-stabilized gold nanoparticles incubated RBC. From this it is clear that, Interaction of nanoparticles with rbc modifies the membrane property like adsorption, penetration, and localization. Such effects affect surface chemistry of RBC [21, 22].

Interfacial tension measurement of Cord RBC suspension using the Pendant drop method

Interfacial tension of umbilical cord RBC was measured using SCA 20 software and the Pendant drop method [23]. Images of umbilical cord RBCs were extracted and fitted with Young's Laplace Equation.

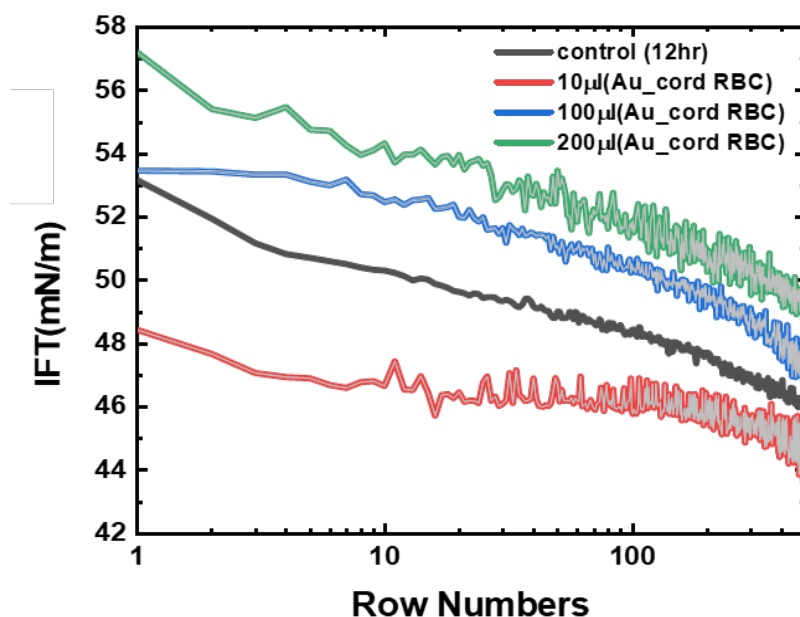


Figure 4: Interfacial tension Measurement of Cord Blood-RBC incubated with gold nanoparticles a) control b) 5 μ l c) 10 μ l d) 100 μ l e) 200 μ l

Interfacial tension of Cord-RBC treated with citrate stabilized gold nanoparticle of volume 10 μ l from 40 nM concentration has a lower value as compared with pristine cord-RBC as shown in Figure 4. However, Cord RBC treated with higher concentration i.e. 100 μ l and 200 μ l of 40 nM gold nanoparticles has high interfacial tension. The IFT of umbilical cord RBC influences the pathophysiology of surrounding medium which depend on the physical state of the RBC membrane.

Conclusion

Variations in umbilical cord RBC were observed in the presence of low and high concentration of citrate stabilized gold nanoparticles viz. 10 μ l and 100 μ l of 40 nM. Reduction in signal intensity is observed in UV-visible and Raman spectroscopy. According to the manuscript, low-dose (10 μ l per dose) AuNPs do not have a significant effect on the spectral properties of cord blood RBCs, although increasing the amount of AuNP from 10 μ l to 100 μ l showed observable changes. The effect of AuNPs on dose dependency is critical for future Toxicity Assessment of AuNPs and development of AuNPs. Furthermore, with a high concentration (100 μ l), the interfacial tension was increased and reduced for 10 μ l of citrate-stabilized gold nanoparticles. The study suggests that lower volumes of AuNP exposure (10 μ l) have no significant impact on the spectral characteristics of cord RBCs. From the current analysis, it is observed that concentration-dependent relationship between AuNP exposure and changes in spectral characteristics of RBC. Henceforth, this study will be useful for evaluating potential toxicity of AuNPs and optimizing AuNP formulation in Umbilical cord RBC.

Author Contributions

Sarika Hinge: conception, design, data collection, Data analysis, results interpretation, validation, and manuscript preparation. Gauri Kulkarni: Validation and supervision. All authors reviewed the Results and approved the final version of the Manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article.

Declarations

Competing Interest: The authors have no relevant financial or non-financial interest to disclose.

Ethics Declarations

Conflict of interest

The authors declare that there is no conflict of interest to declare.

Consent to participate

Not applicable.

Ethical approval

Not applicable.

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