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EXTRACORPOREAL CIRCULATION IN HAEMODIALYSIS DOES NOT IMPAIR RED BLOOD CELLS: EVIDENCE FROM BIOPSY OF BLOOD SAMPLES COLLECTED PRIOR AND AFTER DIALYSIS WITH ADVANCED MICROSCOPES

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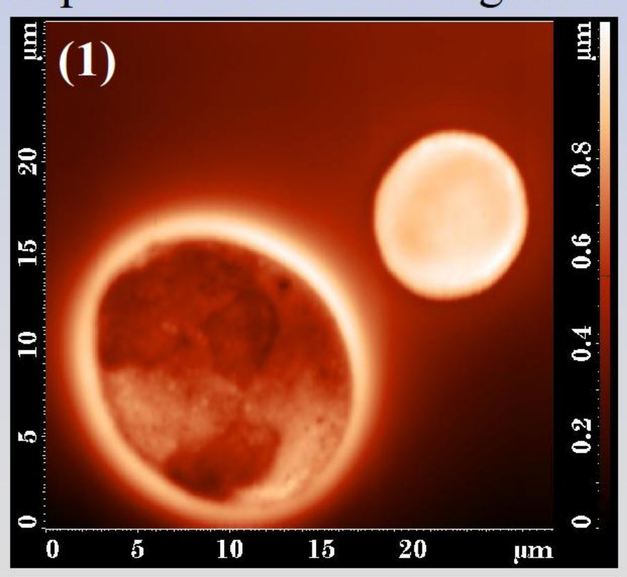
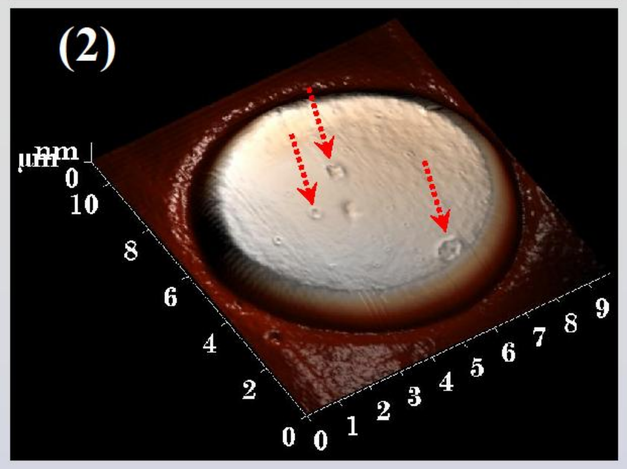
OBJECTIVES: In haemodialysis (HD) blood cells are possibly subjected to both mechanical stress and biochemical activation during the extracorporeal circulation due to the unavoidably imperfect biocompatibility of the employed materials. To resolve information on these issues at the cellular level we surveyed intact red blood cells (iRBCs) of HD patients at the *beginning* and *end* of dialysis.

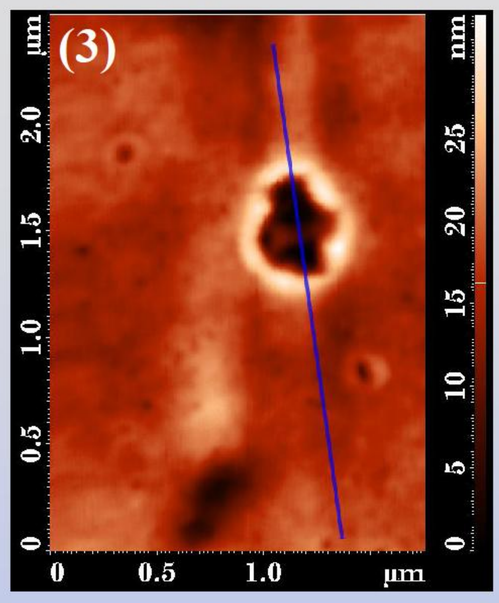
METHODS: We comparatively studied iRBCs collected at the *beginning* and *end* of dialysis (Pre-HD and Post-HD, respectively) coming from 11 HD patients subjected to standard 4-hour dialysis thrice a week under various membranes (polysulfone, polyester-polymer alloy, cuprophan, acrylonitrile-sodium methallylsulfonate copolymer and ethylene-vinyl-alcohol copolymer) with Nikkiso® DBB-06 units. The iRBCs refer to freshly collected RBCs (in EDTA tubes) deposited onto glass slides in single-layered form without farther treatment except for mild centrifugation (120xg, 5 min) for the collection of plasma and reduction of Hct at the value 10%.

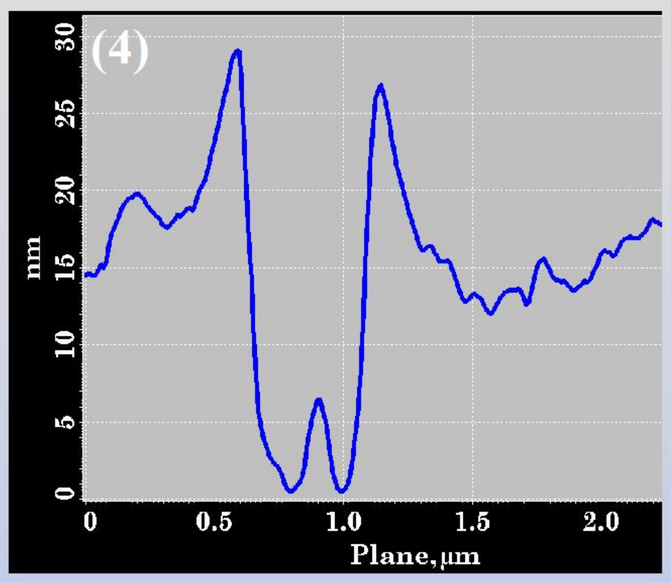
The iRBCs were studied with the Atomic-Force Microscope (AFM) [1-3] and Scanning-Electron Microscope (SEM) that can morphologically survey both entire cells, at the micrometer scale (1 μm=10^{-6} m), Figures (1)-(2), and cell membrane at the nanometer scale (1 nm=10^{-9} m), Figures (3)-(4) [1-3]. In particular, we studied iRBCs collected at the *beginning* (N=306) and *end* (N=305) of the dialysis session. These results are contrasted to data obtained on iRBCs (N=310) of 11 healthy donors as well.

RESULTS: Both the AFM and SEM data show that the membrane of iRBCs displays morphological abnormalities (MA), Figures (2)-(3) [3]. These MA reminisce of circular and linear ulcers, thus they are termed *c*UMA and *l*UMA, respectively [3]. The *c*UMA and *l*UMA have typical size 100-2000 and 500-3000 nm, respectively as shown in Figure (4). Their population counts 5.24 and 0.25 per iRBC, respectively.

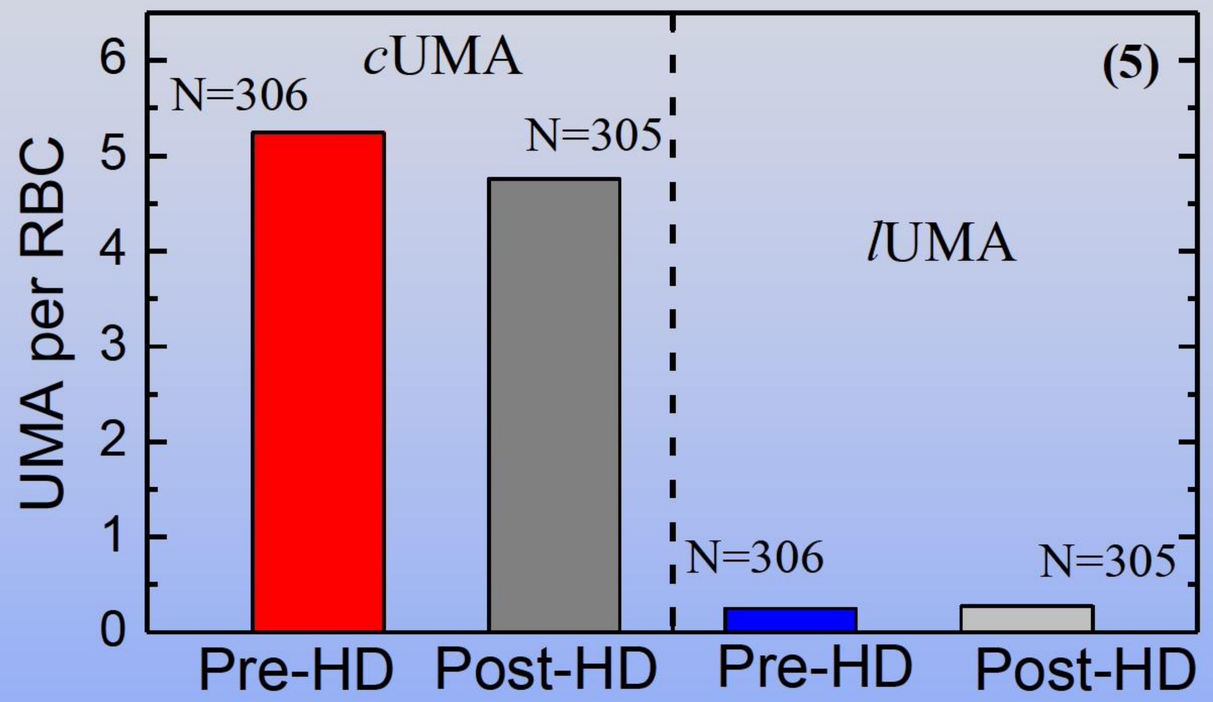








The percentage change of the *c*UMA and *l*UMA population per iRBC during the dialysis session is not statistically significant ($p>0.05$), attaining values -9.2% and 12.0%, respectively, Figure (5). When compared with the healthy donors, the *c*UMA population observed in HD patients presents an intense increase, 55% ($p<0.001$). The *l*UMA population does not present statistically significant ($p>0.05$) difference between the two groups.



CONCLUSIONS: Nowadays, HD has become a mature method in which the employed materials and methods exert only minor, if any, mechanical stress and biochemical activation on RBCs, at least for the typical duration of 4 hours commonly employed in clinical practice. We conclude that the extracorporeal circulation can be safely excluded from the possible mechanisms that influence RBCs and motivate/promote their premature elimination by the reticuloendothelial system, ultimately contributing to chronic anaemia [3].

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