

Extracellular DNA in saliva is protected from cleavage by DNase I

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Background: Extracellular DNA (ecDNA) is mostly analyzed in plasma, but it is present also in other body fluids. Salivary ecDNA is now studied as a biomarker of oral and other cancers. Besides the presence of specific sequences, the quantity of ecDNA might be of special interest for the diagnosis and monitoring of other, especially inflammatory diseases. However, there is no consensus on the pre-analytical processing of saliva samples.

Objectives: The aim of this study was to analyze the effects of centrifugation, freezing and DNase I treatment on quantity and integrity of ecDNA.

Methods: Unstimulated saliva from 20 healthy adults was aliquoted to obtain DNA isolated from saliva centrifuged at 1,600xg and 16,000xg. Aliquots were either used fresh or frozen before isolation. Some aliquots were incubated with DNase I for 30 min before isolation. Concentration of ecDNA was quantified using fluorescence-based dye and real-time PCR. DNase activity was measured using single-radial enzyme-diffusion assay.

Results: Majority of DNA in saliva can be removed by second centrifugation, leaving only low concentrations in the supernatant. Only 2.3 % of total ecDNA remains in saliva after second centrifugation. Freezing had no major effect on ecDNA concentration, but it decreased DNase activity of saliva samples by 35 %. DNase I treatment decreased salivary ecDNA by 80.2 % in saliva centrifuged once. No effect of DNase I treatment was observed in saliva centrifuged twice, suggesting this ecDNA is protected against nuclease activity.

Conclusion: Most of the ecDNA in saliva is removed by centrifugation similarly to other body fluids. This ecDNA is resistant against cleavage. Whether the protection is provided by proteins, small vesicles or the tertiary structures of DNA is unclear. Understanding the physiology of salivary ecDNA holds promise for unraveling its roles in pathophysiological processes and potentially unlocking novel diagnostic or therapeutic targets. Especially, as the absent effect of freezing enables widespread clinical.