DIGITAL RESOLUTION OF FORENSIC BIOLOGICAL MIXTURES PROVIDES SEPARATION OF 100% PURE CELLS FROM EACH CONTRIBUTOR FOR CLEAR-CUT GENETIC ANALYSIS

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Background

Biological mixtures represent a relevant number of all samples reaching the forensic labs. DNA profiling of these mixtures is hampered by the contributions of different individuals. In principle, when distinct individuals contribute with different cell types, their genetic profiles may be obtained by cell separation. However, no purification method reported to date can guarantee an isolation of pure cells belonging to the same class (e.g. epithelial, blood cells, sperm).

Here we show how the use of DEPArray™, an image-based, microfluidic, digital sorter with single-cell resolution, can provide 100%-pure cells for exact genetic profiling of forensic case evidences.

Materials and Methods

Initially the method was defined and tested with simulated forensic evidences, prepared by mixing few microliters [1,5-75 µl] of peripheral blood, semen and saliva from different donors in different ratios, and spotted on fabric, paper or swab and let dry. After storage (1-42 days, mean 17,5), simulated evidences were resuspended, immunofluorescently labeled and loaded on DEPArray™ for cells identification and isolation. AmpFISTR®NGM Select Kit was used to profile recovered cells, single or pools.

The defined workflow was applied to forensic case evidences obtained from three different sexual assaults cases: two small portions cut out from trans-cervical swabs and three victims' garments fragments.

Results

We demonstrated the ability to reliably:

Discriminate between cell types in the mixture (sperm, epithelial or blood cells).
Isolate, from the same biological mixture, multiple aliquots of pure cells (1-50) of each type.
Obtain complete, clean genetic profiles from as low as 5 homogeneous cells for each donor in the mixture, without detectable allelic contamination.

Identify and isolate pure cells from each contributor present in the forensic cases biological evidences and generate from that clear-cut genetic profiles also from tiny amount of starting material.

Highlights

The method was successfully applied to forensic case evidences, and the biological mixtures were resolved by isolating population of pure cells from each contributor; the genotyping provided clean genetic profiles, confirming the reliability of the method and the improvement provided.