Forensic Science International: Genetics Supplement Series 5 (2015) e653–e655

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

A new approach for the separation of spermatozoa from other cell types in forensically relevant samples

F. Grosjean*, V. Castella

Forensic Genetics Unit, University Center of Legal Medicine Lausanne and Geneva, Chemin de la Vulliette 4, CH-1000 Lausanne 25, Switzerland

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 20 August 2015
Accepted 7 October 2015
Available online 20 October 2015

Keywords:
Cell separation
CD52
Magnetic column
Sexual assault
Spermatozoa

ABSTRACT

Due to imbalance in genetic material contribution, gynecological samples collected following a sexual assault are challenging to process in order to resolve the male contributor's DNA profile. We set up a new and fast procedure for the recovery and separation of cells from cotton swabs, or other supports. Using spermatozoa specific CD52 antibody coupled to magnetic beads along with magnetic columns, this procedure was first developed and optimized by flow cytometry. It allows the recovery of two enriched cell fractions: a sperm fraction, mostly enriched with the alleged offender's spermatozoa, and a non-sperm fraction, mostly enriched with cells from the victim. Processing fresh as well as six months old mock samples, made of buccal swabs loaded with sperm dilutions, resulted in full single NGM SElect DNA profiles of the sperm donors, respectively the epithelial cells donors, for the sperm and the non-sperm fractions. Untreated duplicate samples processed in parallel only provided the autosomal DNA profiles of the epithelial cells donors. This new procedure can be rapidly tested and adopted by forensic laboratories worldwide as it uses material already commercially available. Moreover it can be easily automated with existing platform, and could therefore provide a mean to rapidly reduce existing backlogs.

Introduction

The separation of spermatozoa from other cells types is the main concern and the center of interest in the study and processing of gynecological samples collected post sexual assault. Those samples consist mainly in cells originating from the victim and very little amount of cells from the alleged offender. For the vast majority of samples, processing without cells separation will result in the victim's DNA profile only, whereas the alleged offender's DNA profile is looked up.

Several approaches were developed to process these difficult samples, such as differential lyses [1], Y-STR amplification [2,3], laser micro-dissection [4,5], cell sorting [6–9], sieve-based filtration [10,11], micro-fluidic devices [12] or immunomagnetic beads cell separation of fresh samples [13]. While all of these approaches use different principles to separate the cells, none can be easily automated, prevent inter-samples contamination and provide CODIS-submittable autosomal STR profiles from several months, or years, old dry samples.

CD52, also known as the "maturation-associated sperm antigen" is a glycoprotein secreted into the seminal plasma by the epididymal epithelial cells and acquired by the spermatozoa's surface while they travel through the vas deferens [14]. This protein is found on the spermatozoa's head [15] and can be used to selectively stain spermatozoa and differentiate them from epithelial cells.

The aim of this study was to develop a method to recover cells from swabs, stain them with CD52 antibody coupled to magnetic beads and subsequently separate spermatozoa from the original cellular mixture, on MACS magnetic columns (Miltenyi Biotec, Germany), to amplify and detect the semen donors DNA profiles.

Materials and methods

Mock samples were prepared using buccal swabs loaded with PBS-diluted sperm. Swabs were stored up to six months at room temperature. Cells were recovered from the swabs by incubation in 800 μl PBS 2 mM EDTA for 30 min at 25 °C on a thermomixer. The cell suspensions were spun down and the pellets were stained for 15 min at 4 °C in 50 μl PBS 2 mM EDTA 8 μl CD52 antibody coupled to microbeads (REA164 clone, Miltenyi Biotec, Germany). Cells were washed once with 150 μl PBS 2 mM EDTA and resuspended in 300 μl PBS 2 mM EDTA. LC columns (Miltenyi Biotec, Germany) were rinsed twice with 500 μl PBS 2 mM EDTA before the stained cells suspensions were transferred onto them. Non-sperm cells were collected in the flow-through. While retained on the column,

* Corresponding author. Fax: +41 213147090.
E-mail address: Frederic.Grosjean@chuv.ch (F. Grosjean).

http://dx.doi.org/10.1016/j.fsigss.2015.10.013
1875-1768/

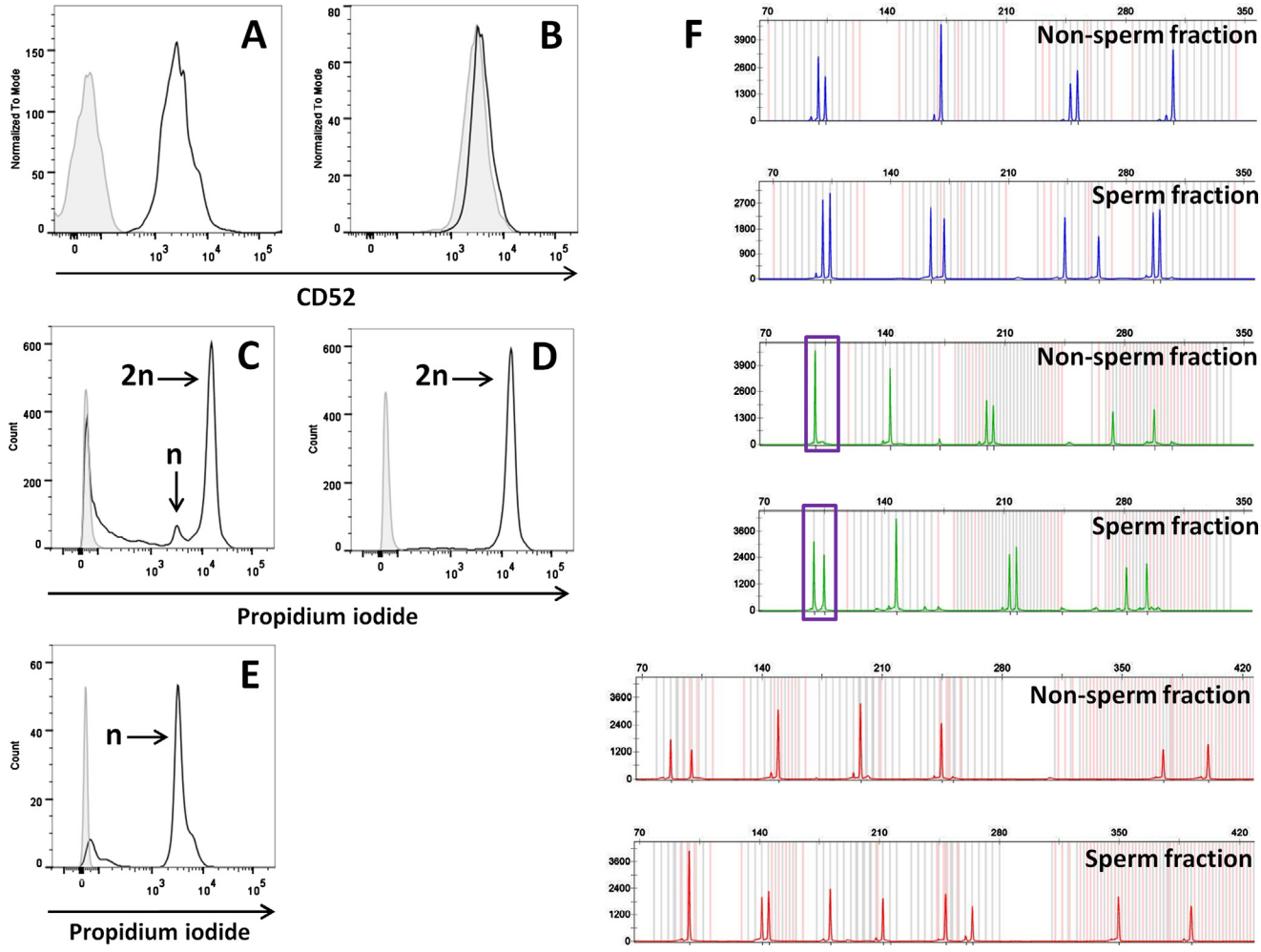

Fig. 1. (A) CD52 stained recovered spermatozoa show a strong shift in fluorescence compared to unstained autofluorescence (grey curve). (B) No shift in fluorescence is observed for recovered epithelial cells compared to autofluorescence (grey curve). (C) Non-separated samples show haploid (n), diploid (2n) peaks and PI negative events. (D) Non-sperm fractions show a diploid PI peak and absence of haploid signal, while sperm fractions (E) show a haploid PI peak. (F) Single male (sperm fraction) and female (non-sperm fraction) DNA profiles were obtained post separation. Boxes indicate amelogenin.

spermatozoa were washed twice with 500 μl PBS 2 mM EDTA. Columns were then removed from the magnetic field and, using 700 μl PBS 2 mM EDTA, spermatozoa were flushed out in new clean eppendorfs. All collected fractions were spun down and pellets were kept in 200 μl PBS 2 mM EDTA prior to cell lysis using QIAamp kit (Qiagen) following the provided protocol. PCR amplifications were performed using AmpFLSTR NGM SElect kit (Life Technologies) according to the manufacturer protocol. Electropherograms were obtained by running the amplification products on a 3130xl Genetic Analyzer from Applied Biosystems. STR profiles were analyzed with Applied Biosystems' Gene Mapper V3.2.1.

Results and discussion

Despite prolonged storage of dry samples, CD52 epitopes were preserved on spermatozoa and enabled specific staining similar to fresh samples. Very low expression of CD52 was found on epithelial cells as shown by flow cytometry (Fig. 1A and B).

Compared to non-separated samples (Fig. 1C), collected fractions post-MACS separation, stained with propidium iodide (PI), showed diploid (2n) peaks for the non-sperm fractions (Fig. 1D) and haploid (n) peaks for the sperm fractions (Fig. 1E) when analyzed by flow cytometry. Successful separations of buccal samples loaded with highly diluted sperm (up to 3000x) were shown by flow cytometry (data not shown).

Using the procedure described above, fresh and six months old buccal swabs loaded with diluted sperm gave fully usable NGM SElect profiles for each of the collected fractions, matching the profiles of the original epithelial cells or semen donors (Fig. 1F). A preliminary study using gynecological samples, taken from two hours up to four days post-coitus, suggests that the procedure described above can be used for real samples. Additional tests are ongoing.

The overall procedure is easy and does not require expensive material to process the samples. The time needed for a separation is comparable to commercial differential lysis kits.

MACS cell separation techniques are already used by a large panel of laboratories worldwide to separate all kind of live cells. With our procedure, this technology can be used to separate dry cellular mixtures. Automation solutions already exist to process several samples in parallel using MACS columns and could be an approach to rapidly process the existing backlogs samples. One patent application was filled in on July 2015 (PCT/EP2015/062838).

Role of funding

No financial support has been received for this work.

Conflict of interest

None.

Acknowledgements

The authors wish to thank all the DNA donors and the members of the laboratory technical team for their precious help, as well as Miltenyi Biotec for providing the microbeads-coupled CD52 antibody.

References

[1] K. Yoshida, K. Sekiguchi, N. Mizuno, K. Kasai, I. Sakai, H. Sato, et al., The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen, Forensic Sci. Int. 72 (1995) 25–33.
[2] L. Roewer, Y chromosome STR typing in crime casework, Forensic Sci. Med. Pathol. 5 (2009) 77–84.
[3] J. Purps, S. Siegert, S. Willuweit, M. Nagy, C. Alves, R. Salazar, et al., A global analysis of Y-chromosomal haplotype diversity for 23 STR loci, Forensic Sci. Int. Genet. 12 (2014) 12–23.
[4] K. Anslinger, B. Bayer, B. Mack, W. Eisenmenger, Sex-specific fluorescent labelling of cells for laser microdissection and DNA profiling, Int. J. Legal Med. 121 (2007) 54–56.
[5] C.T. Sanders, N. Sanchez, J. Ballantyne, D.A. Peterson, Laser microdissection separation of pure spermatozoa from epithelial cells for short tandem repeat analysis, J. Forensic Sci. 51 (2006) 748–757.
[6] W.M. Schoell, M. Klintschar, R. Mirhashemi, B. Pertl, Separation of sperm and vaginal cells with flow cytometry for DNA typing after sexual assault, Obstet. Gynecol. 94 (1999) 623–627.
[7] W.M. Schoell, M. Klintschar, R. Mirhashemi, D. Strunk, A. Giuliani, G. Bogensberger, et al., Separation of sperm and vaginal cells based on ploidy, MHC class I-, CD45-, and cytokeratin expression for enhancement of DNA typing after sexual assault, Cytometry 36 (1999) 319–323.
[8] N. Di Nunno, M. Melato, A. Vimercati, C. Di Nunno, F. Costantinides, C. Vecchiotti, et al., DNA identification of sperm cells collected and sorted by flow cytometry, Am. J. Forensic Med. Pathol. 24 (2003) 254–270.
[9] T.J. Verdon, R.J. Mitchell, W. Chen, K. Xiao, R.A. van Oorschot, FACS separation of non-compromised forensically relevant biological mixtures, Forensic Sci. Int. Genet. 14 (2015) 194–200.
[10] J. Chen, L. Kobilinsky, D. Wolosin, R. Shaler, H. Baum, A physical method for separating spermatozoa from epithelial cells in sexual assault evidence, J. Forensic Sci. 43 (1998) 114–118.
[11] A.M. Garvin, Filtration based DNA preparation for sexual assault cases, J. Forensic Sci. 48 (2003) 1084–1087.
[12] K.M. Horsman, S.L. Barker, J.P. Ferrance, K.A. Forrest, K.A. Koen, J.P. Landers, Separation of sperm and epithelial cells in a microfabricated device: potential application to forensic analysis of sexual assault evidence, Anal. Chem. 77 (2005) 742–749.
[13] X.B. Li, Q.S. Wang, Y. Feng, S.H. Ning, Y.Y. Miao, Y.Q. Wang, et al., Magnetic bead-based separation of sperm from buccal epithelial cells using a monoclonal antibody against MOSPD3, Int. J. Legal Med. 128 (2014) 905–911.
[14] C.H. Yeung, T.G. Cooper, E. Nieschlag, Human epididymal secreted protein CD52 on ejaculated spermatozoa: correlations with semen characteristics and the effect of its antibody, Mol. Hum. Reprod. 3 (1997) 1045–1051.
[15] C. Kirchhoff, CD52 is the 'major maturation-associated' sperm membrane antigen, Mol. Hum. Reprod. 2 (1996) 9–17.