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Research paper

A novel cell culture model as a tool for forensic biology experiments and validations

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ABSTRACT

To improve and advance DNA forensic casework investigation outcomes, extensive field and laboratory experiments are carried out in a broad range of relevant branches, such as touch and trace DNA, secondary DNA transfer and contamination confinement. Moreover, the development of new forensic tools, for example new sampling appliances, by commercial companies requires ongoing validation and assessment by forensic scientists. A frequent challenge in these kinds of experiments and validations is the lack of a stable, reproducible and flexible biological reference material. As a possible solution, we present here a cell culture model based on skin-derived human dermal fibroblasts. Cultured cells were harvested, quantified and dried on glass slides. These slides were used in adhesive tape-lifting experiments and tests of DNA crossover confinement by UV irradiation. The use of this model enabled a simple and concise comparison between four adhesive tapes, as well as a straightforward demonstration of the effect of UV irradiation intensities on DNA quantity and degradation. In conclusion, we believe this model has great potential to serve as an efficient research tool in forensic biology.

1. Introduction

Touch DNA refers to biological material that cannot be attributed to a specific body fluid or tissue and is deposited by direct or secondary transfer from an individual to an item [1]. In recent years, extensive research has been performed to explore the forensic aspects of touch DNA and DNA transfer [1–4]. The main approach to conducting experiments on touch DNA and secondary transfer issues involves the use of DNA deposition by volunteers [3,5], who are usually asked by researchers to handle a certain object or rub their hand against cloth for varying periods of time. The main disadvantage of this method is the significant variance among donors and also between depositions made by the same individual. Some individuals tend to shed high amounts of cellular material in these experiments, while others barely shed detectable levels [6]. The variance can also be high when testing the same individual at different time points, depending on physiological status, behavior prior to the experiment, strength of grip and more. In some cases, DNA not attributed to the donor has been found on an examined item [7], emphasizing another complexity involved in using donors. It is also impossible to control DNA quantity in this kind of model and in some cases, results may be hard to analyze [6]. To obtain meaningful results, researchers usually include a large number of samples from multiple donors, such that both conducting the experiment and analyzing the data require a great deal of time and resources [8]. Studies aiming to solve this problem have not yet provided a satisfactory solution [9,10].

Scientists also use various body fluids, such as blood [11], saliva [3] or buccal swabs [12] to carry out a wide range of tests. The main advantages of these practices include the presence of these body fluids at many crime scenes, their convenient accessibility and the ability to dilute them to the required DNA concentration. However, using this type of biological material for comparing sampling methods [13,14] or for DNA carryover contamination experiments may not be optimal. Since touch DNA is currently a prevalent source of forensic evidence, it is highly reasonable to use a biological material that most resembles the properties of touch DNA in these kinds of studies. This will enable forensic scientists to rely on the results obtained, along with data collected by other means, to make educated decisions when choosing a sampling method or decontamination protocol.

In this study, we present a novel model for performing forensic biology experiments and validations using cultured skin-derived

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human dermal fibroblasts. As a proof of concept, we used this model to answer two questions. First, we scraped the cells onto cloth and compared the DNA sampling efficiency of four adhesive tapes. In addition, we examined the effectiveness of UV irradiation in controlling laboratory contaminations given the increased sensitivity of amplification kits available today. Previously, a leukemic cell line was used to apply defined cell counts on swabs to examine the effect of ethylene oxide treatment on forensic consumables [15]. However, to the best of our knowledge, this is the first time that cultured cells have been used as a model for comparative touch DNA research.

2. Materials and methods

2.1. Cell culture

Primary skin-derived human dermal fibroblast cells (hDF1) [16] were cultured as monolayers in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin streptomycin solution, 2 mM L-glutamine and 10% fetal bovine serum. Cell Petri dishes or flasks were placed in a humidified incubator (37° C, 5% CO_2) until harvest. All cell culture solutions were purchased from Biological Industries, Beit Haemek.

2.2. Sample preparation

Culture medium from hDF1 cell Petri dishes or flasks was removed and pre-heated trypsin-EDTA solution B (Biological Industries) was added. Next, cells were suspended in Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium (Biological Industries) and centrifuged (2000 RPM, 10 min). The supernatant was removed and the remaining pellet was re-suspended in 1 mL DPBS and kept at 4° C. To calibrate final cell quantity on slides according to experimental requirements, a set of serial dilutions was performed. From the 1 mL re-suspension, 50 μL were diluted into 250 μL of DPBS to create a primary fivefold dilution and four more fivefold serial dilutions were made. From each dilution, 50 μL of the solution was placed on glass slides (SuperFrost™, MENZEL™) and left to dry at room temperature. Once dried, slides were sampled as described in the "Tape-lifting" or "Ultra-violet (UV) irradiation" sections in accordance with the intended use of each slide set. A different batch of slides was prepared and used for these experiments. DNA from tapes or swabs was then extracted and quantified as described in the "DNA extraction, quantification, replication and profiling" section. This calibration was done by DNA quantification since cell count by a hemocytometer is prone to error [17] and is not accurate enough for this purpose. In accordance with DNA quantification results and experimental requirements, the original 1 mL re-suspension was diluted with DPBS and 50 μL was placed on each slide (See Fig. 1A). Slides were dried and kept until use. Experiments were performed within 30 days of slide preparation.

2.3. Tape-lifting

2.3.1. Adhesive tape-lifting—cell slide scraping

Pieces of white cloth (20 × 20 cm, 100% cotton) were irradiated on both sides with UV light (10 J/cm^2), placed on a disposable nylon sheet and fixed on a Styrofoam surface with pins. A 6-well plate (Nunc™) in which the bottom part of the wells was removed was placed on the cloth and fixed with pins. The position of each well on the cloth was outlined with a fine marker. The measured surface area of each circle was 9.16 cm^2 (Fig. 1B). Glass slides containing equal amounts of cellular material were prepared as described in the "Sample preparation" section. The slides were placed above the wells and the dried cells were carefully scraped onto the cloth

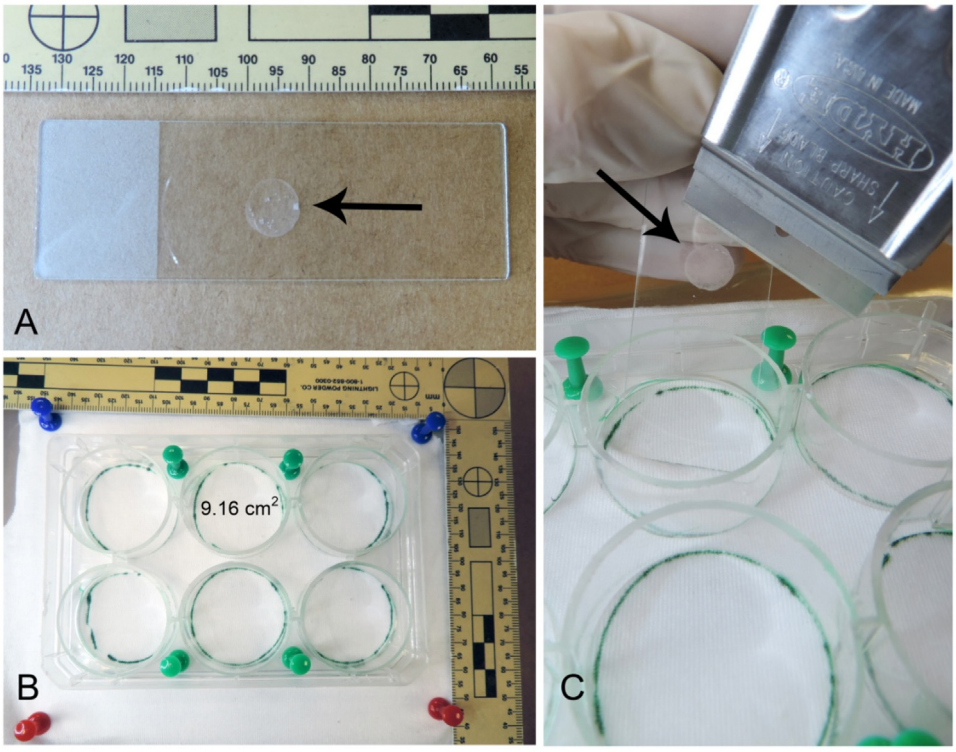

Fig. 1. Experimental model: Cultured skin-derived human dermal fibroblasts (hDF1). A, A glass slide on which 50 μL of hDF1 cell suspension was left to dry. The arrow points the location of the dried cellular material. B, The six-well plate used for tape-lifting experiments on hDF1 cells. The bottom part of all wells was removed and the plate was fixed above a cloth placed on a Styrofoam surface covered with a disposable nylon sheet. The measured surface area of each circle is 9.16 cm^2. C, Scraping dried hDF1 cell into a single well of the six-well plate. After scraping, the material was spread around the well with a sterile plastic rod. The arrow points the location of the dried cellular material.