

Cell-Free DNA: A change in perspective



Green Mountain DNA Conference
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DNA Evidence at the ATF Lab

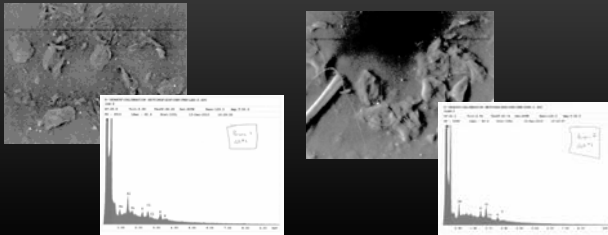
- What we Do
 - Illegal firearms and ammunition
 - Explosives components (pre- and post-blast)
 - Incendiary devices (Molotov Cocktails and wicks)
 - Trace evidence (Hairs)
 - Documents (threat letters and envelopes)
 - Wearer DNA (hats, clothing, etc.)
 - Arson (Wicks, gas cans, etc.)



>90% of our evidence samples are from "touch evidence"

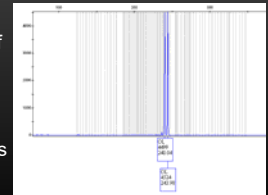
How can we differentiate cells in fingerprints?

X-ray Fluorescence



Mr. Nastr (mRNA STR)

- Epithelial cells filled with keratin
- Therefore, massive quantities of keratin produced toward the end of the cell's life
- Many copies of mRNA possibly present
- Found polymorphic STR sites within the introns of keratin proteins
- Unfortunately, spliced introns don't survive very long



What is a “Shedder”

- March, 2012 – What causes someone to be a shedder?



Shedder



Non-Shedder

or

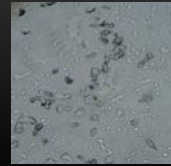


What is a “Shedder”

- First Attempt: Do shedders deposit more cells?
- Fingerprint of known shedder appeared to have approximately the same number of cells as a fingerprint from a non-shedder



50X



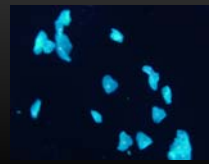
100X

What is a “Shedder”

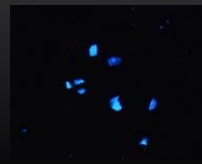
- Second Attempt: Do non-shedders demonstrate a higher level of nuclease activity on the skin surface?
- No

What is a “Shedder”

- Third Attempt: Do shedders deposit more nucleated cells?
- Few if any nucleated cells observed in either fingerprint



DAPI-stained buccal swab smear



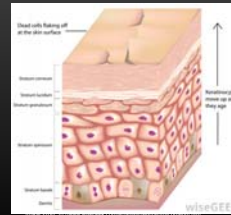
DAPI-stained fingerprint

Shedder

Who cares what a shedder is – where is the DNA coming from in touch samples!



Maturation of the Skin

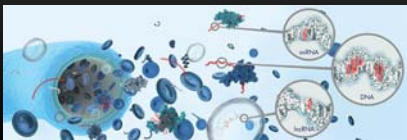


- Cornification of the Stratum Corneum
 - Keratin scaffolding
 - Disintegration of subcellular compartments → loss of nucleus
 - Mechanism unknown

Where does the DNA in a touch sample come from?

Cell-Free Nucleic Acid (CNA)

- Also described in literature as:
 - Extra-cellular DNA
 - Cell-free DNA
- Circulating CNAs (both DNA and RNA) were first described by Mandel and Metals in the 40's
- Not really pursued again until the 70's looking at cancer patients
- Increased importance in 90's in the medical field
- >44,000 publications listed in Pubmed when "cell-free DNA" searched



Taken from Qiagen website

Cell-Free Nucleic Acid (CNA)

- Presence of cell-free nucleic acids suggested previously by Kita et al. (2008) and Linacre et al. (2010)
- Presence of CNAs in sweat confirmed by Quinones et al. in 2012



Cell-Free Nucleic Acid (CNA)

Forensic Science International: Genetics

Presentence and potential of cell free DNA in different types of forensic samples

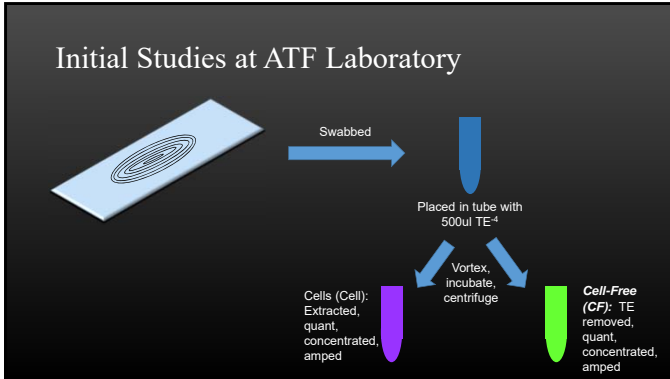
Mads Vandervort, David Van Hooft, Anneke Baasman, Filip Van Nieuwenburgh, Dieter DeMaesseneer

Optical characterization of epidermal cells and their relationship to DNA recovery from touch samples [version 1; referees: 2 approved]

Cristina E. Stanciu, M. Katherine Philpott, Ye Jin Kwon, Eduardo E. Bustamante, Christopher J. Ehrhardt

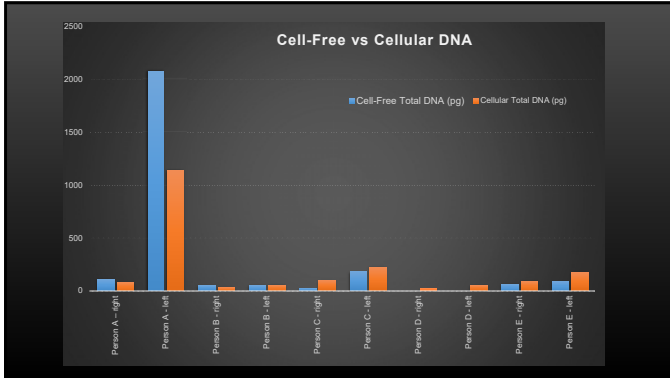
Source of Cell-Free DNA

- Still unclear
- Sweat?
- Leaked from keratinocytes?
- Other?

Initial Studies at ATF Laboratory

	Cell-Free Total DNA (pg)	% CF of Total DNA	Cellular Total DNA (pg)	% Cell of Total DNA	Total DNA (cellular and CFD)
Person A - right	111.44	58.70%	78.4	41.30%	189.84
Person A - left	2079	64.52%	1143.1	35.48%	3222.10
Person B - right	49.42	58.88%	34.51	41.12%	83.93
Person B - left	53.9	49.55%	54.88	50.45%	108.78
Person C - right	19.236	17.08%	93.38	82.92%	112.62
Person C - left	179.9	44.39%	225.4	55.61%	405.30
Person D - right	0	0.00%	17.08	100.00%	17.08
Person D - left	0	0.00%	52.29	100.00%	52.29
Person E - right	65.45	42.06%	90.16	57.94%	155.61
Person E - left	88.97	34.21%	171.08	65.79%	260.05



Collaborative Study with the Defense Forensic Science Center (DFSC) and George Washington University

- Laboratory work performed by Brittany Fickau during a summer internship
- Similar sample prep as described previously except index and middle fingers used to deposit prints
- Five different individuals
- Three components analyzed:
 - Cellular
 - Cell-free
 - Swab
- All components concentrated to 10ul and entire volume amplified

GWU/DFSC Initial Results

Donor	L/R	Cellular Alleles	Cell Free Alleles	Swab Alleles
A	L	0	12	4
	R	1	21	N/A
B	L	16	28	N/A
	R	1	18	17
C	L	0	20	0
	R	0	2	N/A
D	L	1	3	1
	R	2	4	N/A
E	L	7	30	N/A
	R	6	20	5

- Variation between each individual
- More alleles detected in the cell-free component for each individual

Second Round of Testing

- Sample prep and collection the same as above
- Prints collected from the right and left hands of three individuals at ten time points

Variation over time

Percent Profile Detected from Cellular Fraction			
Hand/Day	F	G	H
L1	3.33	0	100
L2	0	0	53.33
L3	100	0	0
L4	0	0	0
L8	0	0	0
L9	10	0	0
L10	6.67	0	0
R1	0	3.33	10
R2	3.33	0	0
R3	33.33	0	0
R4	0	0	0
R8	0	0	0
R9	0	3.33	0
R10	6.67	0	0

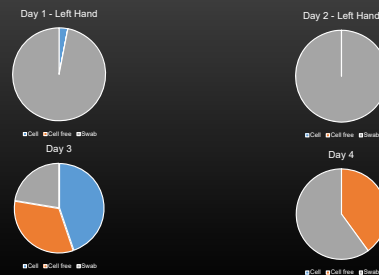
Variation over time

Percent Profile Detected from Cell-Free Fraction			
Hand/Day	F	G	H
L1	0	0	26.67
L2	0	0	80
L3	73.33	96.67	0
L4	16.67	3.33	0
L8	0	0	60
L9	0	0	3.33
L10	3.33	0	0
R1	0	6.67	0
R2	0	0	56.67
R3	0	80	53.33
R4	26.67	3.33	0
R8	0	0	36.67
R9	20	0	10
R10	26.67	0	0

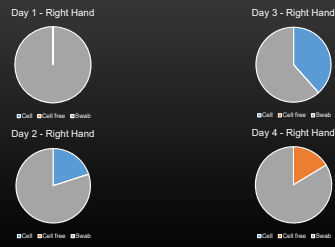
Variation over time

Percent Profile Detected from Swab Extraction			
Hand/Day	F	G	H
L1	43.33	80	100
L2	56.67	13.33	66.67
L3	50	13.33	16.67
L4	36.67	3.33	0
L8	0	0	0
L9	0	0	66.67
L10	66.67	0	0
R1	36.67	6.67	73.33
R2	13.33	0	10
R3	46.67	3.33	100
R4	83.33	0	0
R8	0	0	0
R9	0	0	73.33
R10	50	0	0

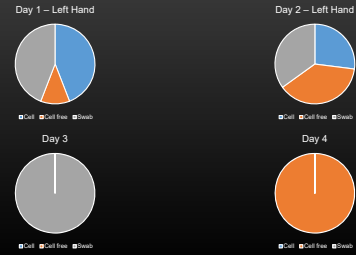
Second Round of Testing – Person 1



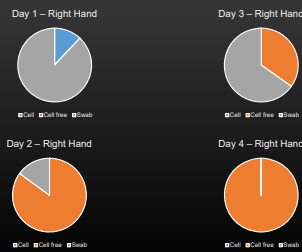
Second Round of Testing – Person 1



Second Round of Testing – Person 2



Second Round of Testing – Person 2



Secondary Transfer – Foreign Alleles

Foreign Alleles	# Detected	Total Alleles	Percent Foreign
Cellular	2	102	1.96
Cell-Free	11	176	6.25
Swab	76	401	18.95

Swab Type

Cotton Swab		
Fraction	Total Alleles	% of Swab Alleles
Cellular	102	25%
Cell-Free	176	44%
Swab	401	

Nylon -Flocked		
Fraction	# Alleles	% of Swab Alleles
Cellular	21	13%
Cell-Free	18	11%
Nylon Swab	166	

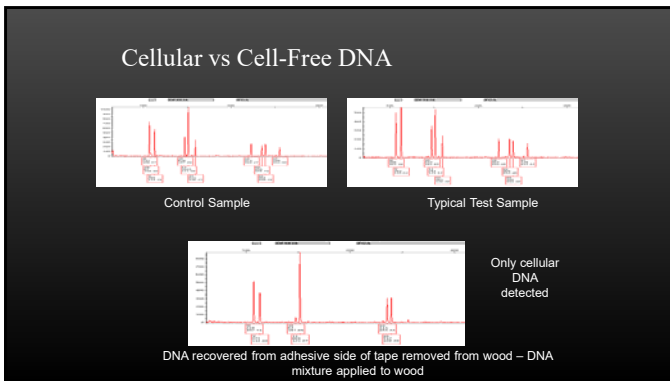
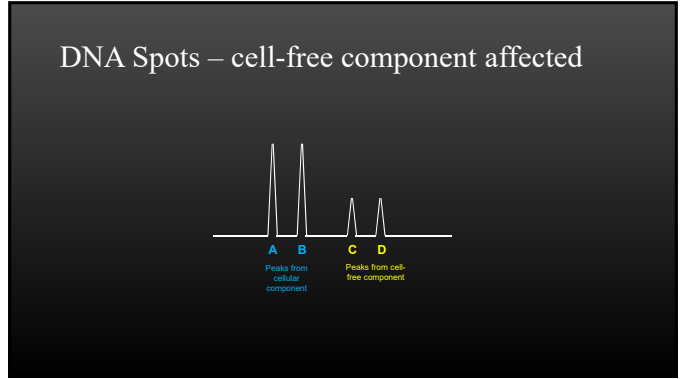
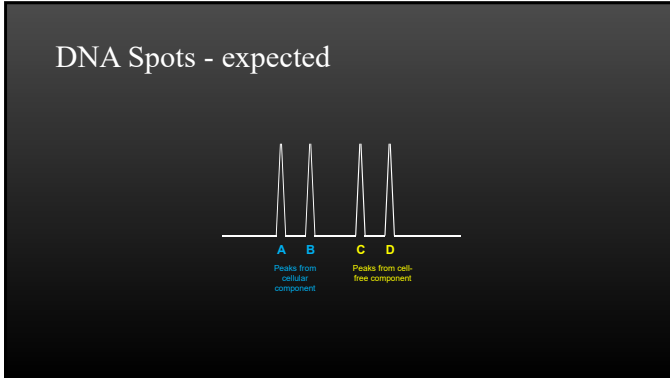


Many research studies attempting to improve the recovery of DNA utilize cells – what if cell-free DNA behaves differently?

Effects of Substrate

- Studied the recovery of cellular DNA and cell-free DNA from various substrates
- Created a mixture of cells from Person A and purified DNA from Person B with an expectation of equal recovery
- Aliquots of this mixture deposited on various substrates (e.g. PVC, rubber, wood, paper, galvanized steel, tape) and allowed to dry overnight at room temperature





What is causing the loss?

- Of the substrates studied, only wood or paper (wood product) affected

Other Reasons

- Extraction method



Other Reasons

- Direct amplification
- Different rates of degradation
- Environmental insults
 - Copper
 - Bacterial
- Secondary transfer
- ESDA
- Better swabbing/collection devices
- Quick and easy way to collect protein and DNA from same sample
- We know swabs retain DNA – is it cellular or cell-free?
- Tape-lifts: is cell-free DNA collected or just cellular?

Conclusions

- Cell-free DNA is present in fingerprints
- The quantity and relative proportion of cell-free DNA compared to cellular DNA is variable
- The source is unknown
- Consider using both cellular and cell-free DNA when conducting research on touch evidence samples
- Very little research in the forensic field on this topic
- Cell-free DNA most likely exists in most/all the body fluids we test

Acknowledgements

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