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## ACCOMPLISHMENTS:

### □ Purpose of the project:

The NIJ funded program 2015-DN-BX-K042 started in April 2016 and the team of GE Global Research and University of Akron proposed to develop photo-degradable materials and swab protocol development to enable technology for light-initiated dissolvable swabs for improved recovery of forensic samples. The purpose of the program was to develop novel photo-dissolvable swab materials that function as regular swabs during sample collection of aqueous samples that can be completely degraded after exposure to DNA-friendly longwave UV light, thereby enabling both high collection efficiency and maximum release for low-abundance DNA samples.

### □ What was accomplished under these goals?

The team successfully down-selected 2 photo-degradable material compositions, and using a polymer-blending approach along with form factor improvements, formulated electro-spun fibers with improved wettability, bio-specimen pick-up and sample release into the PCR workflow upon photo-degradation. In the 1<sup>st</sup> year the U Akron team focused on developing photo-degradable materials, and GE Global Research focused on testing the photo-degradation kinetics, implementing the materials in PCR workflow to study inhibition of PCR and finally the materials were down-selected based on the performance. In the 1<sup>st</sup> year GE also demonstrated that the photo-dissolvable swabs (at wavelength of 365 nm) did not inhibit PCR reactions. The DNA was not affected during photo-degradation of PHEMA-NBS (100%, 50% and 25% functionalized polymers), nor were the byproducts formed inhibitory to PCR reaction and subsequent analysis. The degradation wavelength is selected as a good compromise between reduced sensitivity to sunlight, and potential DNA damage during the irradiation process. In the second year, U Akron worked on scaling up the down-selected material and GE Global Research worked on developing electro-spun compositions, developing protocols, and successfully enabling formulation of photo-degradable fibers that were rolled onto Q-tip or spun onto to aluminum foils to enable mat format for downstream PCR analysis. GE also developed protocols for sample placements and pick-up into the mats/swabs. Additionally, GE also developed a "prototype" UV-breadboard light set-up for faster degradation of the photo-dissolvable polymer and the light fixture was eventually used to develop the PCR workflow.

### Project findings:

#### Overall summary

- Synthesized and investigated 3 different photo-degradable polymer systems (PVA, PHEMA, PHEA systems) as potential swab materials. (U Akron)
- Photocleavable polymers (N-bromo succiniamide (NBS) system down-selected with good photo-degradation efficiency and appropriate UV wavelength down-selection for photo-degradation has been accomplished (GE-GRC)
- The down selected UV wavelengths (365nm, 385nm) shows no evidence of any DNA damage (GE-GRC)
- Polyhydroxyethyl acrylic (PHEMA) polymer system, and its photodegraded byproducts shows negligible PCR inhibition even when the Polymer:DNA is as high as 50,000:1 (GE-GRC)
- GE Global Research found successful protocol to electro-spin photo-degradable polymer material into fibers especially using a polymer blending approach. Successful trials were performed with PEG and PVP polymer additives.

- Experiments are conducted with ng scale DNA and showed modest release from the electrospun fiber materials upon irradiation.
- Release efficiency from the blending approach the team accomplished the ability of trace sample (<5ng) pick-up and release efficiency of only up to 70% after UV irradiation.
- The down-selected photo-degradable polymers don't show complete dissolution in pure water. This may be due to unintended (and preventable) crosslinks in the raw material used (being confirmed now). Dissolution conditions with the present material system was only achieved with blending the photo-degradable polymer with a water-soluble blend mixing approach.
- With proper mixing of fully functionalized photodissolvable polymer with PEG we optimized the electrospin form factors and mechanical integrity of the swab/mat materials so that they do release <5% in control samples (from 30-40% in the past) that were not subjected to irradiation.
- Further optimization needed of the photo-degradable materials, thicknesses of swab/mats for mechanical integrity for pick-up and complete dissolution on light application, and high throughput method development for mass manufacturing of swabs with consistency and reproducibility.
- Cells workflow Optimization protocol that we have developed that photo-dissolvable swabs can be used to pick up and release cells without any impact on the workflow at any stage.

### **Methodology:**

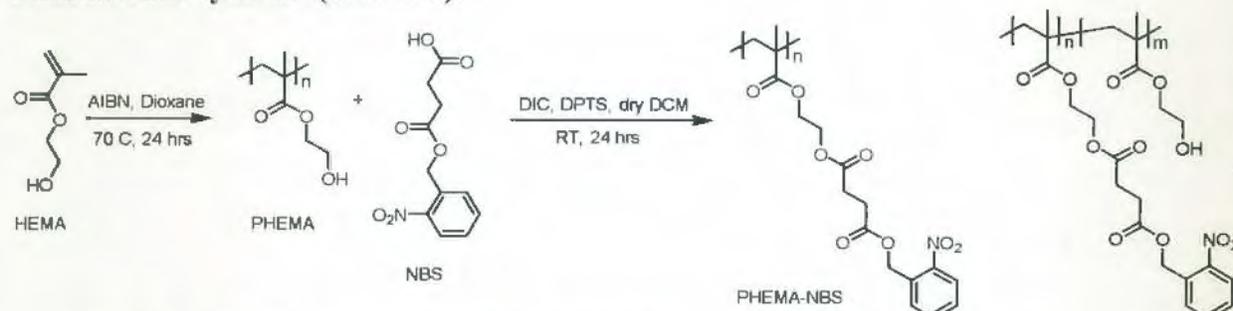
The approach of DNA pick-up/release the team sought after was in developing a “water insoluble” polymer (to be made into a swab) which can become soluble by two approaches: 1) photo-degradation into small oligomers or monomers that are water soluble, or 2) photo-degradation of surface groups/side chains of the polymer exposing the underlying aqueous soluble polymer. After acquisition of the sample, photo-irradiation of the swab will cleave the photo-dissolvable moieties, thus providing monomeric and/or oligomeric products that are more hydrophilic and enabling their dissolution into the processing buffer along with the biological sample. This process enables dissolution of the entire polymeric coating along with the specimen. To this end, the team was unable to produce approach-1 polymers that did not affect DNA, but synthesized several approach-2 polymer systems and down-selected 2<sup>nd</sup> approach polymers. The polymers made by that method showed success with no PCR inhibition. So further development and optimization was performed on approach-2 polymers to accomplish remainder of the grant proposal tasks. Approach-1, which still hold promise, was put on hold. Further discussion in the summary below will only focus on the down-selected materials as representative examples and its data analysis, results, and conclusions. For details on the overall compilation of work please refer to our previously submitted reports.

### **Results:**

#### Task 1—Develop and test photo-dissolvable polymers

*Synthesis of photo-dissolvable polymers.* The first polymer constitutes photo-cleavable pendant groups that can be tagged on to a water soluble polymer backbone. Three polymers, polyvinyl alcohol (PVA), polyhydroxyethyl acrylate (PHEA) and polyhydroxyethyl methacrylate (PHEMA) are being investigated. Two photocleavable moieties are being investigated thus far, nitrobenzyl succinate (NBS), a derivative of nitrobenzyl alcohol, and a trimethoxy alkoxyphenacyl compound.

### PHEMA-NBS Synthesis (Scheme 1):



As shown in the scheme 1, hydroxyethyl acrylate was polymerized under conventional radical polymerization conditions and subsequently NBS was conjugated to the PHEMA. As determined by NMR, 100%, 50% or 25% of the hydroxyl groups were functionalized to provide PHEMA-NBS. Two batches of such conjugated polymers were prepared, one with molecular weight ( $M_w$ ) of 70 kDa and the other with 22 kDa  $M_w$ . The methacrylate version has a higher modulus and this conjugate was prepared anticipating that the acrylate version may have a modulus too low for fabricating into fibers or films. All the synthesized compounds were characterized by  $^1\text{H}$  NMR-spectroscopy and GPC analysis. Initial photo-degradation were conducted to determine the efficiency of photo-degradation (Figure 1). About 10 mg of PHEMA-NBS were dissolved in 3 mL of dichloromethane. The solutions were placed in quartz test tubes and irradiated at 350 nm for 30 minutes before characterization.

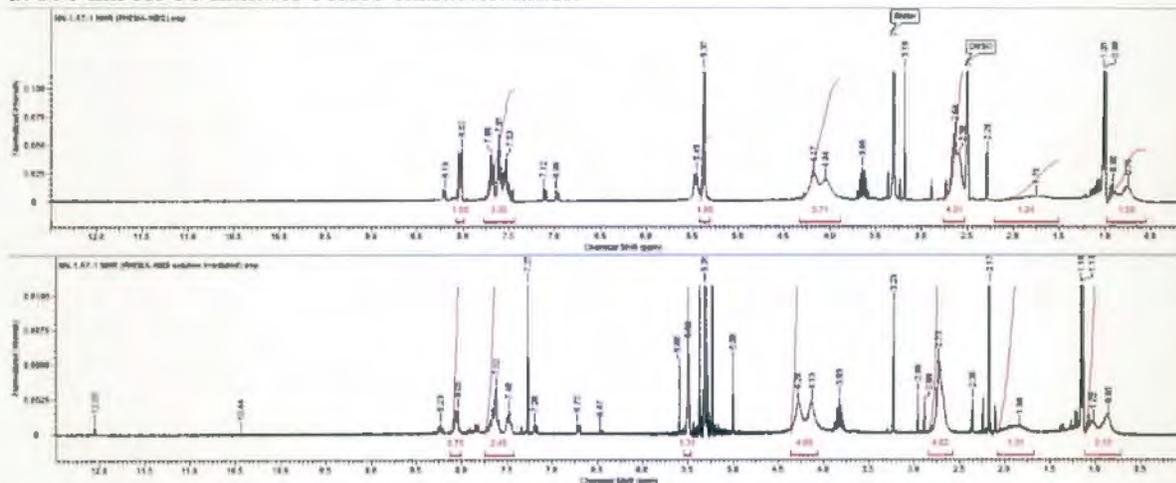


Figure 1: Photodegradation studied and characterized by  $^1\text{H}$  NMR analysis

The top spectrum shows the polymer before irradiation while the bottom spectrum shows the polymer after irradiation. After irradiation, peaks corresponding to carboxylic acid and aldehyde can be seen at 12 and 10.5 ppm, respectively. Correspondingly, decrease in peaks corresponding to the parent NBS is seen at 7 to 8.5 ppm and the peaks at about 5.5 ppm. From these integration values, it can be determined that about one fourth of the NBS groups on PHEMA-NBS were cleaved. The presence of extraneous peaks and the reduction of integration values belonging to NBS indicate that cleavage of this group occurs at 350 nm. Figure 2 shows before (left) and after (right) irradiations of the pHEMA-50% NBS in water. The opaque nature becoming clear solution with most of the material going into solution

suggests photo-degradation followed by photo-dissolution of the polymer. We have determined that up to 0.5mg/mL goes into the solution upon photo-irradiation.



Figure:2 Photo-irradiation of PHEMA-50%-NBS subjected to 30 mins (365nm) irradiation in water.

Task 2—Evaluate and down-select photo-dissolvable materials— work conducted at GE

We evaluated precursor materials that are used to make the photo-dissolvable swab to see if these chemical components will inhibit PCR reactions. The purpose of this study is to evaluate and down-select materials that are safer and don't inhibit PCR. This would not only enable quick screening and down-selection of chemistries that would pass the PCR tests without having to spend time and effort to wait until the swab materials are made. The precursor materials (1-4) depicted in Figure 3 were dissolved in DMSO to make stock solutions with concentration of 1mM. Three different dilutions were made in water from stock (0.1 mM, 0.01 mM, 0.001 mM). Also, the experiments were simultaneously conducted with PCR (AmpliTaq assay) and the results are presented in Figure 4. In both the qPCR and PCR reactions there was no inhibition of chemicals 1-4 with final concentration of 4 uM (1:100) and below in Quantifier assay.

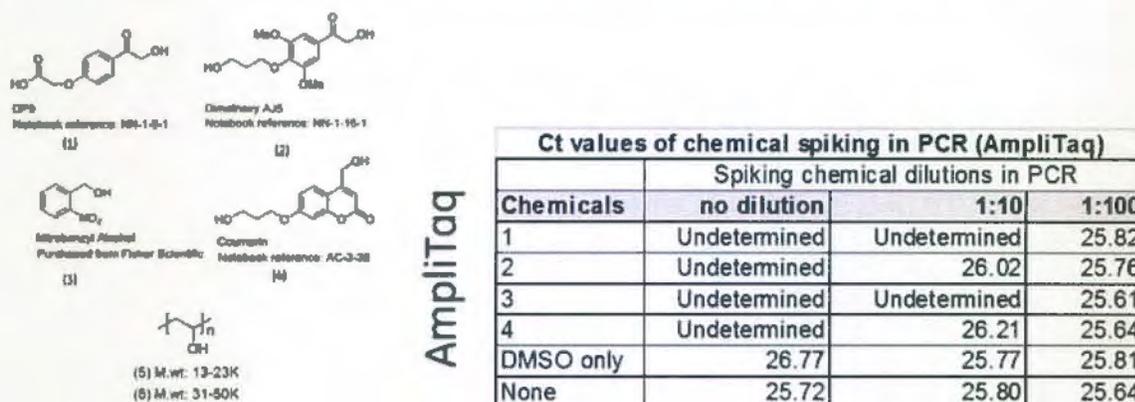


Figure 3: Chemical structures of Monomers and Polymer precursors of swab material

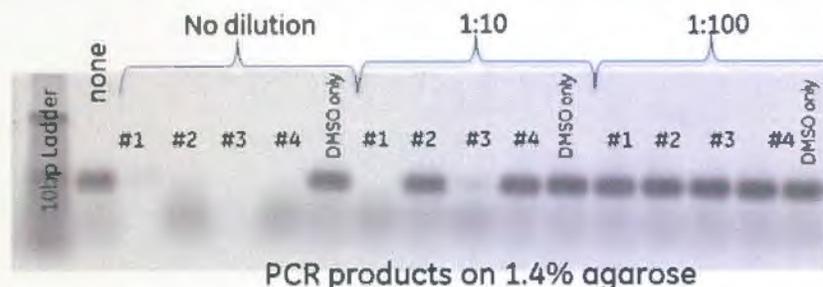


Figure 4: AmpliTaq PCR analysis of Monomers (1-4)

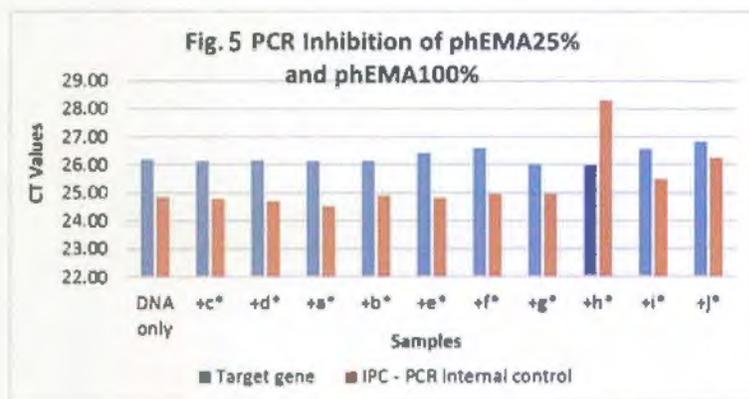
**PCR inhibition tests on photocleavable polymer swab materials.**

To test the capacity of PHEMA in PCR and dissolution/solubility of byproducts in the PCR reaction mixture a lower and a higher derivatized photocleavable group along the side chains of the poly HEMA with derivatized % of 25% (PHEMA25%) and 100% (PHEMA100%) were tested. The tested samples were prepared per Table 1. 100ul of each of the solutions shown below were placed in a clear pyrex glass vial was exposed to 365nm wavelength Luzchem Rayonet photoreactor with measured intensity of 4.5mW/cm<sup>2</sup> when the sample is placed at a distance of 4.5" from the light bulbs and irradiated for 20 minutes.

Table 1. Irradiation samples (365nm/30mins)

Label	Polymer name	Polymer Conc. in water	DNA concn	Notes
a*	PhEMA25%	0.5mg/mL		
b*	PhEMA25%	2.5mg/mL		
c*	xx	xx	xx	DMSO control for tube a*
d*	xx	xx	xx	DMSO control for tube b*
e*	PhEMA25%	0.5mg/mL	5ng/ul	pre-mix
f*	PhEMA25%	2.5mg/mL	5ng/ul	pre-mix
g*	PhEMA100%	0.5mg/mL		
h*	PhEMA100%	2.5mg/mL		
i*	PhEMA100%	0.5mg/mL	5ng/ul	pre-mix
j*	PhEMA100%	2.5mg/mL	5ng/ul	pre-mix

Figure 5: The CT values of target gene among these samples were no much difference in comparing positive control. However, a notable increase of CT scores of IPC from phEMA100% at a higher concentration of 2.5 mg/ml (h\* and j\*, orange bar) suggested it might reach the capacity limit in PCR. [\*samples subjected to UV irradiation prior to PCR analysis]



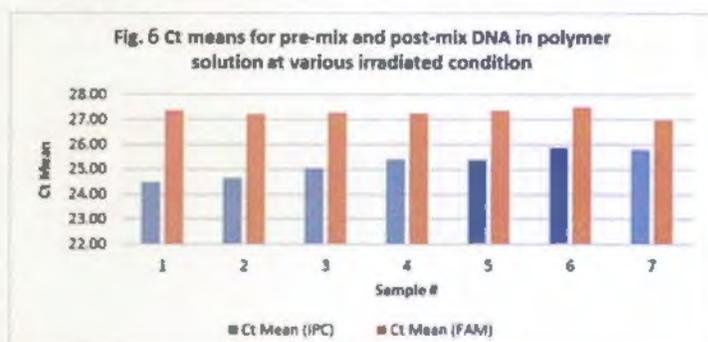
### Wavelength dependence and Irradiation light source down-selection:

To choose appropriate power density and wavelength for a) improved photo-degradation kinetics and dissolution/solubility of the byproducts in the PCR mixture and b) test the effect of chosen UV wavelengths towards PCR inhibition the following experiments were performed. Three different light sources were screened, LED/365nm, LED/385nm and Rayonet/365nm with measured light intensities 26mW/cm<sup>2</sup>, 25.4mW/cm<sup>2</sup> and 4.83mW/cm<sup>2</sup> respectively when the samples were placed at a distance of 4" from the light source. A 50mg/ml stock solution of PhEMA-NBS100% was made by dissolving the polymer in DMSO, then suspended in water to make testing solution. The testing solutions containing pre-mix DNA or none (100 ul) were irradiated by LED/365nm, LED/385nm and Rayonet/365nm for 30 minutes respectively,

then spiked into PCR. Solutions that were irradiated using the both the high-power density LEDs (365nm and 385nm) showed slight yellow coloration suggesting prolonged irradiation and close to complete polymer degradation than the lower power density Rayonet/365nm. The qPCR results (Table 2 and Figure 6) indicated that there was insignificant inhibition towards DNA by any of the above irradiation conditions (i.e. light wavelength, power intensity, treatment time, or degraded polymer residues).

Table 2: Experimental protocol for Irradiation

PCR Sample#	Irradiation condition	Experiment Description	IPC - internal control	Report gene
1	n/a	10ng DNA+water, positive control	24.49	27.34
2	LED/365nm/30min,	irradiated, then add DNA 10ng	24.64	27.21
3	LED/385nm/30min	irradiated, then add DNA 10ng	25.05	27.25
4	Rayonet/365nm/30min	irradiated, then add DNA 10ng	25.40	27.23
5	LED/365nm/30min,	premix 10n DNA, then irradiated	25.41	27.34
6	LED/385nm/30min	premix 10ng DNA, then irradiated	25.87	27.49
7	Rayonet/365nm/30min	premix 10ng DNA, then irradiated	25.79	26.96
8	n/a	water only, negative control	24.57	Undetermined



### Task 3: Scale-up and Swab Development

Scale up of the chromophore and polymer synthesis has been successfully achieved. The NBS chromophore can be synthesized in one step with minimal purification required. Batches over 30 grams have been synthesized with near quantitative yields. All the three electro-spinning of the PHEMA-NBS (100%, 50% and 25%) functionalized polymer systems either have high hydrophobicity (PHEMA-NBS-100%) or too easily wettable nature (PHEMA-NBS-50% and 25%) and the electro-spun samples either gave too brittle fibers or did not yield any fibers respectively. GE team attempted a polymer blending approach to solve this issue by blending other water soluble systems like PVP, PEG to make the composition electro-spinnable into fiber system that has improved strength and enabled mat and swab formats to be developed that was used in the downstream workflow. In a typical experiment, 500 mg of polymer was dissolved into 2 mL (2:3 DMF-CHCl<sub>3</sub>) and the material was taken into 3 mL syringe. The target (aluminium foil, a conductive substrate) was placed 12cm from needle. The automated syringe was set at a flowrate of 1 mL/hr and syringe tip-22Gauge; voltage-21-25kV. After most of the 2 ml was pushed out onto the foil the aluminium foil was removed for further studies and the electro-spun material for pulled out of aluminum foil for further studies.



Figure 7: GE-GRC's electro-spinning device and microscopic image of swab fibers.

A high-power chip scale package (CSP) 385nm UV LED system that exposes the swab from 4 sides was set-up to illuminate and maximize light output for Swab dissolution as shown in Figure 8. Thermal transfer material encapsulation of the LEDs extends their lifetime, and allows high power operation with Diode operating conditions of 12.8V and 4.5 Watts. A LED driver board was chosen to allow automatic current adjustment as operating conditions change due to the LED junction heating up during operation. The UV LED Breadboard - System was tested using a Thermal Imaging system to monitor the LED temperature and to ensure safe operating conditions (see Figure 8, right). A first test with photo-dissolvable swab material indicates that this system can achieve the same performance as the commercial, high-cost unit previously used in a quarter of the time, due to shorter wavelength and higher power.

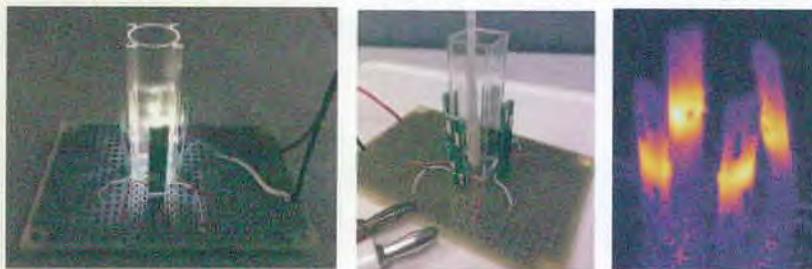


Figure 8: UV LED Breadboard – first version with 385nm. LEDs in operation (left); system test with swab inserted (middle); infrared image of operating LEDs to test heat-sink capability.

During the first test of the UV-LED swab irradiation system the power supply was manually switched on and off in intervals, in order to not exceed the maximum temperature capabilities of the LED. To automate the process, a low cost (<\$25) programmable timer was built for UV-LED light box. Upon pressing the start button, a pre-programmed UV-pulse sequence is activated (Figure 10). This ensures reproducibility and user-friendliness. An on-board LED indicates when UV LED is switched on, the on/off pattern can be programmed using switches directly on the PCB. As next steps we tested the photodissolution of PHEMA-50%-NBS+PEG=1:1 electrospun swab using the UV illumination apparatus described above. This high intensity apparatus showed modest photoillumination using 365nm lights in 6 mins which is 10x faster than conventional low intensity Rayonet photoreactor system which took close to 1hr (Figure 9). However, since the photodegradable system developed in this proposal did not go to complete photodissolution due to material limitation the illumination system couldn't achieve quantitative photodissolution. We plan to continue to evaluate new high-power UV-LEDs (and new semiconductor lasers) available in the market, and envision that much more powerful and efficient systems could be built in near future which can not only accelerate photoreaction to a few seconds but also can enable photodissolution of thicker swabs/mats depending on the formats developed for different forensic workflows. Installing/testing a shorter wavelength high-power LED to accelerate the dissolving of photo-swabs made with the new polymer mixture, will enable our goal of dissolving it completely in a few (<5) minutes (Figure 10).



Figure 9: Photodissolution of PHEMA-50%-NBS+PEG=1:1 swab material.



Figure 10. Custom-built 365nm 10W LED UV-irradiation instrument for photo-swab including power-supply (left) and detail showing sample holder and LED-head enclosure (mounted to heatsink).

### DNA recovery measurement by PCR

**Sample picking methods:** We investigated two approaches for the DNA isolation for PCR evaluation. In method-1, we placed required amounts of Promega DNA solution directly onto the swab and the sample was dried before further studies. In method-2, the DNA was “swabbed” by the swab or mat from the dry surface and placing the Promega DNA solution onto a glass substrate surface and drying it on the glass substrate before swabbing. Quantifiler™ Human DNA Quantification Kit, a TaqMan real-time PCR based kit is designed for the quantification of amplifiable human DNA that is present in a sample. This kit was used to measure DNA released from polymer films with and without irradiation procedure. Typically, 2 ul supernatant from +/- irradiation DNA deposit sample solutions as well as controls (DNA polymer mixed samples and DNA only sample solutions) were spiked into 25 ul PCR reactions. PCR was performed at a standard condition (95°C for 10 minutes; 95°C for 15 seconds, 60°C for 1 minutes, 40 cycles). The quantity of detectable DNA was estimated by using standard curve method. Spely, the DNA recovery rate was calculated by dividing the amount of detectable DNA from the quantity of input DNA. The DNA recovery rate from control samples (with irradiation, orange bar; non-irradiation, blue bar) was plotted in Fig. 11. It appeared that over 90% of DNA was recovered from all the control samples regardless with or without polymer mixing and with or without irradiation using method-1 strategy, suggesting no DNA damage in irradiation and no PCR inhibition from both polymers.

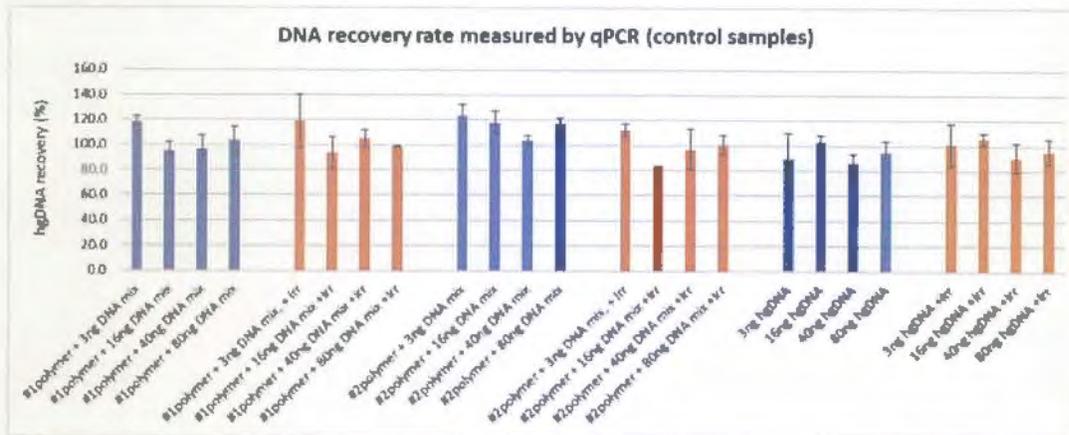


Fig. 11. % trace amounts of DNA recovery rates from qPCR of different polymer blends and functionalized polymers.

The DNA recovery rate from DNA deposited samples (with irradiation, orange bar; non-irradiation, blue bar) was plotted in Fig. 15. Highest DNA recovery rates were obtained from 80 ng DNA deposit samples and moderate rate from the lower (3ng) DNA deposit samples. No significant difference was seen of DNA recovered from polymer substrates by irradiation method, however, all recovery rates were nearly perfect.

In the case of method-2 the results were more encouraging when it comes to the comparison between the % recovery from before and after irradiation. This study was done by mixing 9 ul of 50 ng/ul of hgDNA (Promega #G147A) and 1 ul of 1:50 diluted food dye, placing the sample on a glass substrate. Then 0.5 - 1 mg of fiber from pHEMA-NBS50%+PEG swab, wet with 1 ul of water and then swabbing the dried DNA spots. The picked-up fiber was placed in a vial in 20 ul HET buffer. The results of PCR DNA recovery studies before and after irradiation is shown in Figure 12. Since even the control DNA from the Qiagen Kit showed only %recovery of 30%, the total net %recovery from spun fiber before irradiation was calculated to be 40% while after irradiation sample gave up to 70%. It is notable that the FLOQswab gave quantitative recovery compared to our photo-dissolvable swab. However, we later realized that the FLOQswab is too hydrophobic and therefore keeps the substrate right on the surface without any interaction with the swab interior and won't be an applicable solution in crime scene settings where collection and storage is a necessary condition. We also have reports from end-users that FLOQswabs do not pick up biological materials well, but do elute them well.



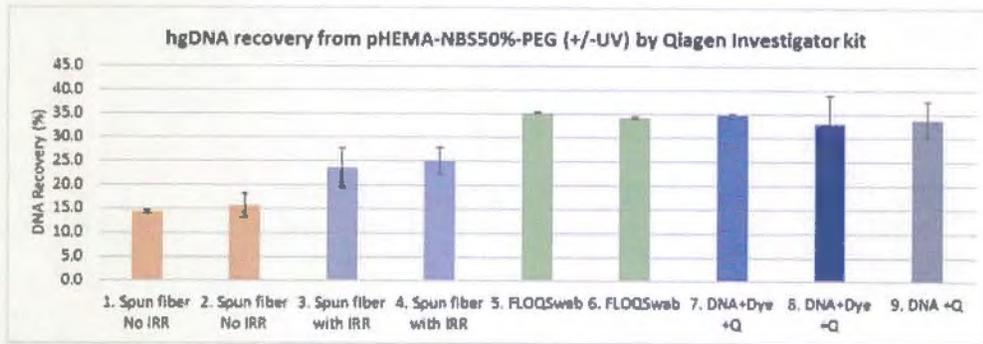


Fig. 12. % trace amounts of DNA recovery rates from dried DNA on different polymer blends and functionalized polymers.

We also performed some preliminary testing on the materials electro-spun on mat formats (Figure 13). Circular 6mm punches were cut and subjected to PCR and the obtained similar release efficiencies (~65% DNA recovery) on 3 ng DNA pick/release studies to that of swabs.

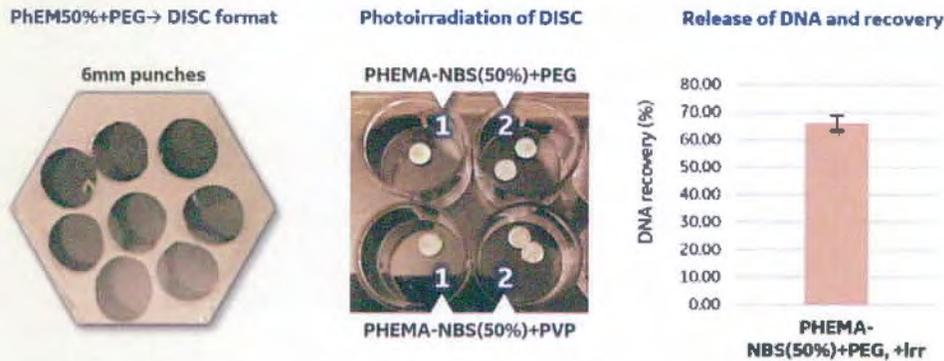


Fig. 13. % trace amounts of DNA recovery rates from dried DNA placed on mat format.

To improve the mechanical integrity of the photodegradable polymer we electrospun the 1: 4 ratio of PHEMANBS(100% functionalized): PEG blend and the fibers were rolled onto a plastic Qtip. Figure 14 shows comparative hgDNA recovery of control swab and the irradiated swab showed upto 5% DNA release on the control swab against 23% on the photoirradiated version. DNA @ 10ng/uL extracted from PhEMa polymer with Qiagen DNA investigator Kit is plotted. Qiagen Investigator kit uses column purification and the column binds DNA as a result recovery is low.

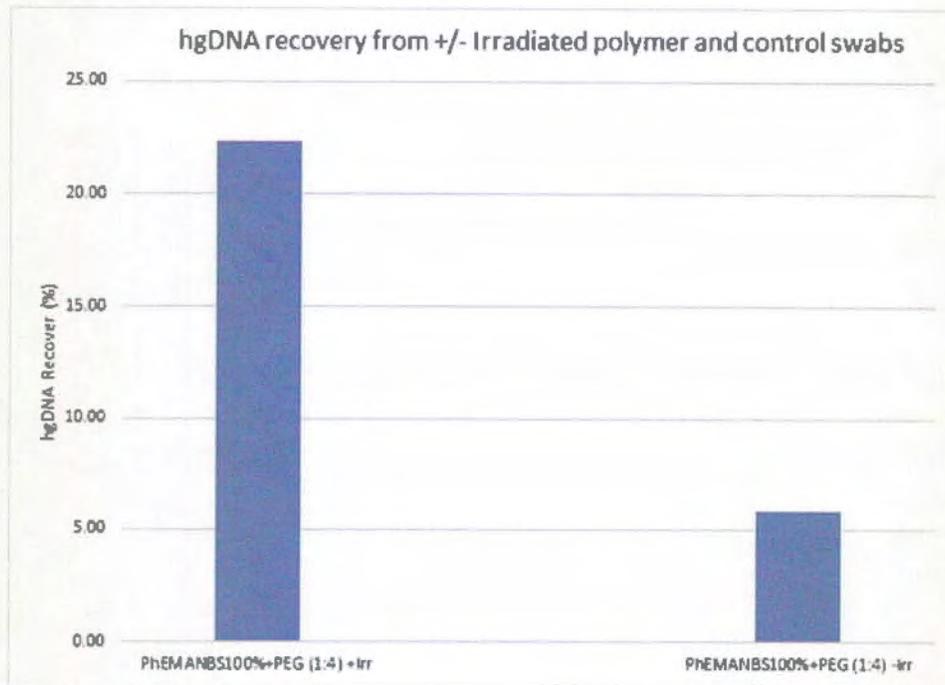


Figure 14:hgDNA recovery from before and after (20mins) irradiation in Rayonet reactor (365nm) of PHEMANBS100%+PEG=1:4 blends.

**Cells workflow Optimization protocol:**

10µl of T-cells pipetted on to Parafilm and air dried for two days. Sample is picked with premoistened photo dissolvable swab, cotton swab (2uL of water to premoisten) and air dried for a day (Figure 15). Swabs were processed through Qiagen protocol that comprises of cell lysing and releasing DNA. We had also included cells in solution as controls to benchmark the amount of DNA released.

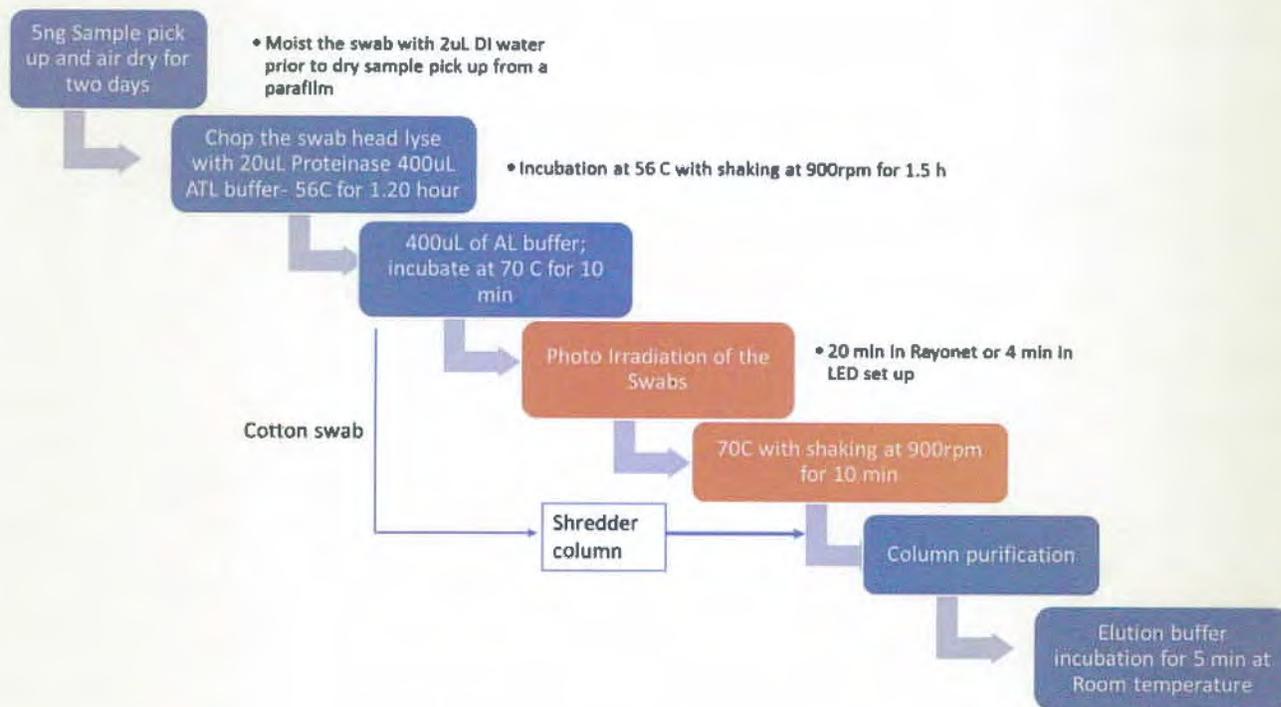


Figure 15: Qiagen workflow is shown in figure. Photo dissolvable swabs need two additional steps when compared with cells in solution and to get maximum recovery out of cotton swab, it need an additional step of using shredder column.

Photoirradiation was performed with Custom-built 365nm 10W LED UV-irradiation system. Samples were irradiated for a period of 4 min with this rapid photoirradiation system. Qiagen mini amplification kit Cat# 56504 was used for extracting DNA. Quantifier™ Human DNA Quantification Kit, a TaqMan real-time PCR based kit is designed for the quantification of amplifiable human DNA that is present in a sample. This kit was used to measure DNA released from polymer films with and without irradiation procedure. Typically, 2 ul supernatant from +/- irradiation DNA deposit sample solutions as well as controls (DNA polymer mixed samples and DNA only sample solutions) were spiked into 25 ul PCR reactions. PCR was performed at a standard condition (95°C for 10 minutes; 95°C for 15 seconds, 60°C for 1 minutes, 40 cycles). The quantity of detectable DNA was estimated by using standard curve method. Results are summarized below in Figure 16,

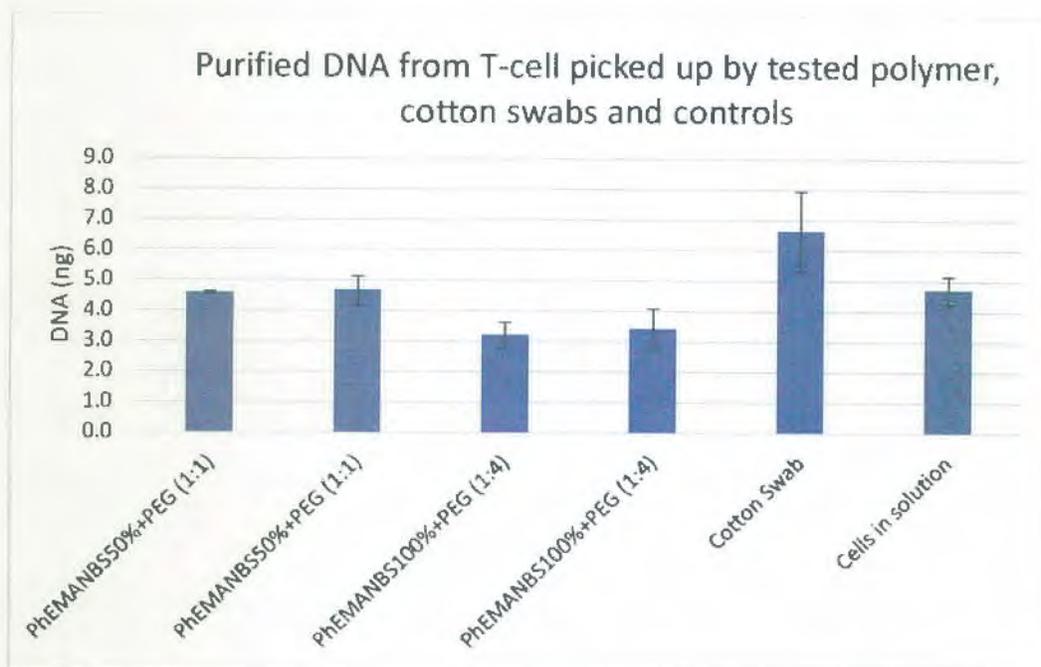


Figure 16: Purified DNA from T-cell picked up by test polymer cotton swabs and controls

Clearly the amount of DNA recovered with PD (photo dissolvable swab) is comparable to that of DNA from cells in solution which is our control. Higher DNA recovery amount with cotton swab is probably due to the additional step of using shredder column in our workflow which is not the typical forensic workflow and also variability in sample pipetting. We have clearly demonstrated that PD swabs can be used to pick up and release cells without any impact on the workflow at any stage.

In conclusion we have shown that photodissolvable polymers can be electrosun into mats/swabs when blended with polymers such as PEG and PVP. We have also shown that polymers release DNA upon shining 365nm light and this technology works not just with known amount of DNA and also cells.

### Conclusion and next steps:

Under grant 2015-DN-BX-K042, the GE Global Research and U Akron team developed PHEMA-NBS polymer swab materials for photo-dissolvable swab approach to perform efficient capture and release of trace samples. These polymers were photo-degradable however they were not directly electro-spinnable into fibers and have solubility limitation of only 0.5mg/mL in water. In order to overcome these limitations, GE used a blending approach with electro-spinnable water soluble polymers and showed that they can be made into swabs and discs. Water soluble polymer blending helped improve the hydrophilicity and fibrous strength of the resulting electro-spun polymer. The team has successfully demonstrated development of photo-dissolvable swab materials and showed progress toward converting the materials into electro-spun fibers with a blending approach and showed initial proof of concept into making swab and mat formats to enable trace analysis pick-up and release with modest efficiencies (up to 70% or perhaps more). However,

there are some critical to quality parameters that need further materials and method development and optimization to enable commercial viability of the method and incorporation of the technology into existing forensic workflows such as replacing the cotton Q-tip for sample collection and release. These improvement areas to be addressed are as follows, a) Photo-degradable materials that offer complete and quantitative photo-dissolution post irradiation in a minimum amount of PCR reaction liquid, b) Method development and optimization of form factor of swab with good mechanical stability, wetting properties and efficient pick-up of biospecimen and retainment on the swab for robust storage until the sample is exposed to appropriate light for release of trace in PCR workflow. To maximize the recovery further it is crucial to optimize the % mixing of blend to polymer and also the thickness of the material onto Swab/paper formats with good mechanical integrity. c) optimize the electro-spinning conditions (including adding additives to mimic cotton like robustness and consistency) to fabricate swabs in a manufacturing environment and d) develop large scale electro-spun grids for high throughput and mass production of swabs. In future with more funding we strongly believe that further optimization of the material system can allow us to improve the material performance, swab fabrication, robust mechanical integrity for pick-up and tailored swab thickness efficient release (>90% of sample).

**Implications for criminal justice policy and practice in the United States:**

Our business unit, GE Healthcare, have been excited by the progress we have made on this NIJ funded program. Further material development and optimization is necessary from our end to make an effective technology transfer and convert this technology into a product. This includes preparing a material with the correct form factor for pickup and release of biological specimens, protocols for materials synthesis, more refined equipment for light activation to dissolve the swab, electro-spinning conditions, and toll vendors for material scale-up needs to be further developed. GE Global Research will work towards achieving these goals and GE Healthcare is waiting on completion of the major tasks before starting full commercialization efforts. This will likely occur in 2019-20 if a robust marketing strategy supports such an effort.

We have not contacted Crime labs for testing the new format at this point . We believe that more work on optimizing materials, protocol, and method development for large scale manufacturing of swabs are the next steps, and if successful will have numerous practical implications on the forensic analysis of samples containing nucleic acid. Most importantly, Low Copy Number (LCN) analysis work will benefit from the efficient collection/release strategy we are developing. Despite the current modest efficiency, the protocol promises to be rapid, and since it does not require a centrifugation step, is easy to automate, miniaturize, and integrate into next-generation DNA analysis instruments such as the RapidHit from IntegenX or the Netbio's DNAscan. Also, a facilitated workflow for blood-swabs can be envisioned: Placing a swab containing the sample (or a small section of it) into a tube with a minimal amount of buffer or water, exposing it to UV light, and using concentrated DNA solution directly in PCR is conceivable; possibly even immersing the swab into the PCR mix followed by UV irradiation and amplification.