Graphene in Textiles for Vector Protection

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ABSTRACT

Arthropod transmitted diseases can have a significant impact on Military readiness. To limit exposure to disease carrying arthropods, the US Military uses the Department of Defense (DoD) Insect Repellent System, which calls for proper wear of a treated uniform, using topical repellent on exposed skin, taking prophylaxis, and sleeping under a bed-net. Both the US Army and Marine Corps use insect repellent treated uniforms. These treatments do provide an initial level of protection to the Warfighter but degrades with laundering and wear. With the rising threat of vector-borne diseases, there is a need to enhance protection to Warfighter through improved uniforms.

Work completed by Brown University showed that dry graphene or graphene oxide nano-films interfered with host-seeking behavior of Aedes aegypti *mosquitoes. The specific aim of this effort is to expand upon this knowledge and to develop military-specific textile technologies to reduce exposure to arthropod bites without degrading the desired properties of uniform materials.*

Early efforts demonstrated proof of concept of two approaches: 1) a polymer-based process was demonstrated by loading various levels of graphene within nylon 6,6 and pressing into films; and 2) the print-based process used a syringe printer to print graphene pastes on the back side of untreated fabric. Efficacy testing was conducted by tracking mosquito landings and bites on the samples.

Currently there are difficulties with the extrusion of fibers for the polymer-based process and the data is inconclusive due to the low graphene loading level in the successfully extruded fiber. The printing-based approach is proving successful as the graphene print patterns afforded a significant reduction in the number of bites as compared to a control fabric, however, in terms of landings, the data is more variable, but trending towards reductions in landings.

The effort aims to development and incorporate graphene into textiles to reduce mosquito attraction while meeting the current physical and spectral requirements of military uniforms. This approach to vector protection coupled with the current uniform treatments will not only meet a defined capability but enable the refinement of current requirements to offer advanced / improved vector protection to the Warfighter.

Reduction in arthropod bites = Reduction in disease = Reduction in lost time = Increased operational readiness and combat effectiveness = Mission completion

***Keywords**: graphene, textile, vector, protection, mosquito, uniform, polymer, print*

1.0 INTRODUCTION

Current uniform treatments are not providing the level of vector protection needed for the Warfighter, especially after wear and laundering. Additionally, as pyrethroid resistance continues to rise and spread, the treatments will become even less effective. As potential battlefronts and strategies are defined, and with the rising threat of vector-borne diseases, there is a need to enhance protection to the Warfighter through improved uniforms / materials that ensure efficacy against resistant mosquito populations and throughout the wear life. Introducing effective and non-insecticidal technologies, such as graphene, into uniforms will ensure that the Warfighter maintains a consistent level of protection through extended wear.

1.1 Background

Arthropod transmitted diseases can have a significant impact on Military readiness. An algorithm published in Military Medicine found that twenty-eight (28) of the top forty (40) endemic diseases impacting military operations were arthropod-borne [1]. The US Military uses personal protective measures to reduce exposure to arthropod-borne diseases. Specifically, the Department of Defense (DoD) Insect Repellent System calls for proper wear of a permethrin-treated uniform, using topical repellent on exposed skin, taking prophylaxis, and sleeping under a bed-net to limit exposure to disease carrying arthropods.

The DoD Insect Repellent System relies on the individual Warfighter to take the steps necessary to protect from vector-borne diseases, however, these steps are not always executed by the Warfighter. Factory treatment of the uniforms with permethrin or etofenprox takes one piece out of the Warfighter’s hands, but there are limitations to this form of protection when it is solely relied upon. Active ingredient loss occurs with wear and laundering, resulting in reduced protection over time. Additionally, pyrethroid resistance is emerging in many mosquito species of concern. This resistance will decrease the effectiveness of the insecticides, requiring higher quantities for protection [2]. As resistance spreads to different areas or becomes even more prominent in already resistant mosquitoes, current protection methods and dose levels will lose effectiveness.

Work at Brown University explored the use of graphene oxide nano-films for mosquito bite prevention. The study showed that when tested against *Aedes aegypti* mosquitoes, dry graphene or graphene oxide nano-films interfered with host-seeking behavior by preventing the mosquito from detecting molecular attractants on the skin [3]. It is also unclear if the films were adhered to the fabric or just placed beneath for testing. While a nano-film approach may have potential for the commercial market, it will not meet the stringent needs of the US Military. In a time when the Military is looking to lighten the load and enhance soldier performance, the application of an additional film barrier to military textiles may increase soldier burden. An additional film barrier (even at the nano-level) will add weight to the overall garment, especially if bonding agents are required to permanently attach the films. It could also reduce breathability and moisture wicking while degrading other desired material properties such as tear and breaking strength. This effort aims to determine if graphene will truly inhibit mosquito host-seeking and if the technology can be made ‘military-centric.’

Additionally, with pyrethroid resistance on the rise, there is potential that currently available treatments (which are all pyrethroids) will cease to protect the Warfighter at the expected and necessary levels. There is a continuous need to research new technologies to combat resistance and bring them into the toolbox to ensure the best protection. Introducing effective and non-insecticidal technologies into our uniforms will ensure that the Warfighter will maintain a consistent level of protection with extended wear as well as against resistant mosquitoes. If successful, incorporating a non-insecticidal technology into current uniforms would provide a huge breakthrough in providing protection from vector-borne diseases.

1.2 Objectives

The objective of this project is to develop military-specific uses for graphene in textiles to reduce exposure to arthropod bites while not degrading the desired properties (physical and spectral) of the uniform materials.

Vector protection is a requirement for both Army and Marine Corps uniforms and is currently accomplished using insect repellent treatments on the fabrics or uniforms, specifically pyrethroids. Although these treatments do provide an initial level of protection, that protection degrades with laundering and wear. Additionally, with pyrethroid resistance on the rise, these treatment methods are at risk of becoming even less effective in the future. Combining these treatments with a technology that reduces mosquito host-seeking behavior will greatly enhance Warfighter protection in the future.

To potentially reap the vector protection benefits of graphene with minimal increase in soldier burden, this effort will explore the incorporation of graphene into uniform textiles using two different methods:

- Incorporation of graphene into textile polymers that can be made into textile fibers.
- Screen printing graphene onto the back of textile fabrics.

2.0 APPROACH

This effort used two different approaches: polymer-based, and print-based. The major focus was to demonstrate that graphene dispersed into a polymer or printed onto a textile fabric affects the ability of mosquitoes to find a blood meal and that graphene can be applied to fabrics using different methods. The work is described below.

2.1 Polymer

Two different types of graphene-loaded polymer pellets (masterbatches) were purchased from Global Graphene Group (Dayton, OH): an electrical grade consisting of 10% by weight of graphene (EGr) in nylon 6, 6 and a mechanical grade consisting of 1% by weight of graphene (MGr)in nylon 6, 6. The two masterbatches were loaded with graphene consisting of different particle sizes. The particle size for the EGr masterbatch was approximately 7µm while the MGr masterbatch used an approximately 4µm particle size. In addition, 100% nylon 6,6 was purchased from PolyOne.

An iterative process was used for this approach, starting with compounding various graphene loading levels into nylon 6,6 and pressing into films, followed by perforating the graphene pressed films, and lastly fiber extrusion. Various pressed nylon 6,6 films containing dispersed graphene were prepared from these materials for testing at USDA. The following polymer compounds were pressed into films: 10% EGr, 5% EGr (compounded from 10% pellets), 1% EGr (compounded from 10% pellets), 1% MGr, and 100% nylon 6,6 (control). Pressed films were perforated using a laser to allow skin volatiles to excape in an attempt to mimic fabric interstices during testing.

Additionally, graphene fiber extrusion was performed both in-house and via contract with the Textile Technology Center (TTC) at Gaston College in Belmont, NC.

2.2 Print

Pre-made graphene pastes were purchased from Global Graphene Group and from Dycotec Materials, while base pastes and binders for an in-house graphene paste formulation were procured from Sigma Aldrich.

A Voltera V-One Syringe Printer was used to print on the back side of untreated (no insect repellent) Defender M fabric. Ultimately, printing will transition to screen printing, however, for the purposes of broadly evaluating pastes and patterns, syringe printing is a much more cost-effective solution. To start, the printing utilized a full coverage approach to evaluate ease of printing and efficacy. Full coverage printing was followed by various patterned prints and coverage areas to identify patterns that offer promising mosquito testing performance for scaled up work with screen printing.

Four principle geometries were selected for patterned prints: dots, grids, lines, and hexagon. For each of these geometries, several patterns were created to vary both the percentage of the area covered by printed materials (e.g., 25%, 50%, and 75%) and the feature size (e.g., diameter for the dot geometry and line width for the grid and line geometries). The selected patterns were printed on the back side of untreated Defender M fabric using the Voltera V-One Syringe Printer for efficacy evaluation. Ultimately, only the Dycotec paste was selected to continue evaluation to include various loading levels of graphene within the paste. The graphene properties of the Dycotec paste are shown in Table 1.

Table 1: Dycotec paste graphene properties.

Graphene Type	Multi-layer
Carbon content	≥ 99%
# of graphene layers	9 ± 6
Flake thickness	3 ± 2nm
Density	200 ± 20 g/L
Surface area	130 ± 5 m^2/g

2.3 Testing

Efficacy testing was conducted in a dry and wet state at USDA-CMAVE. All efficacy testing was conducted using human volunteers.

The specimens were tested against two lab-reared / disease-free mosquito species (*Aedes aegypti* and *Anopheles albimanus*). These mosquito species are used for standard military bite protection testing, as they are both disease carrying vectors of interest and have different biting behaviors. *Aedes aegypti* transmission includes, but is not limited to, yellow fever, Zika virus, and dengue, while *Anopheles albimanus* is the main carrier of malaria. The specimens were tested as described below.

The USDA Minimum Effective Dosage (MED) Test was modified for this effort, as sample size and exposure was consistent with our needs. To prevent biting on the non-test area, the test volunteer wore a latex glove over the hand, a nylon stocking over the arm, and a plastic hook and loop sealed sleeve over the entire forearm with a window cut out approximately 4 cm X 8 cm to allow mosquitoes access to the test swatch. Test samples were either cut or supplied at a size of approximately 9 cm X 14 cm so that a 4 cm X 8 cm rectangle could be accessible through a small window on a loop sealed sleeve (vinyl sheeting) to allow mosquito biting pressure. The test specimen was placed across the window and held in place with masking tape.

The test volunteer placed the prepared arm into a screened cage with approximately 100 female mosquitoes for 3 minutes. During the test period, the test volunteer tracked landings and bites using a hand counter. Data was pooled for both mosquito species tested and presented as the average number of landings or bites in a 3-minute window. Wet state testing was conducted by wetting the test specimen with water using a spray bottle. A minimum of 4 replicates for every treatment type was performed to determine the potential bite / landing reduction from mosquitoes. Over the course of the entire study, a total of 60 control replicates were performed across 3 human subjects, with an average biting rate of 17 ± 1.4 (SEM) per 3-minute interval. The average number of landings in the 3-minute window for the control was 78.4 ± 3.7 (SEM). Therefore, the variability was small, and the number of replicates performed allowed for adequate evaluation of the most successful treatments.

3.0 RESULTS AND DISCUSSION

3.1 Polymer

Due to the nature of the pressed polymer films, mosquitoes were unable to bite through. Therefore, only landing counts were used to determine efficacy of the graphene-loaded polymer films. As shown in Figure 1, landing counts indicate that higher levels of graphene may inhibit mosquito landings, as the 10%wt and 5%wt EGr samples had fewer landings on both wet and dry specimens as compared to the 1%wt EGr and the Nylon 6,6 control. The 1%wt MGr had similar dry landing counts as the 1%wt EGr polymer despite being much thicker. While differences in thickness may account for some of the variation, the 5% wt EGr was thinner than the control, but did show a marked improvement in number of landings, especially when dry. The 1%wt EGr film showed similar landing counts as the control despite being much thinner. All samples exhibited higher landing counts on the wet verses the dry specimens.

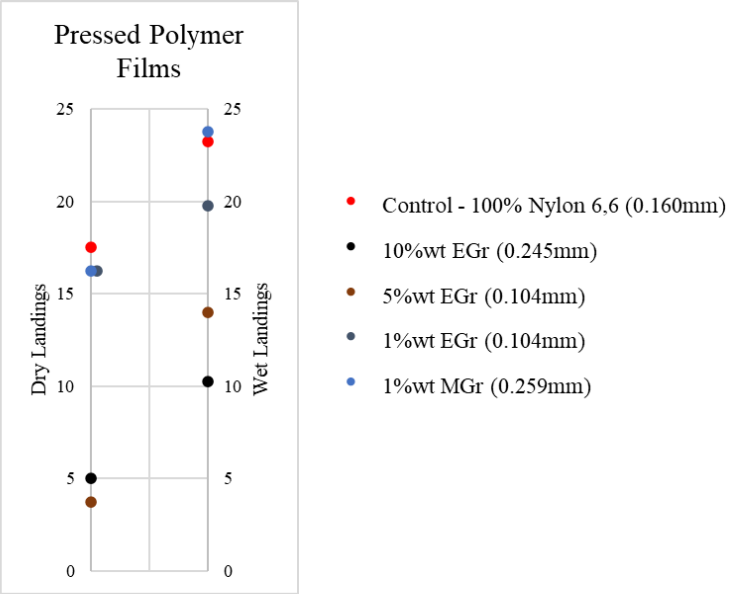


Figure 1: Mosquito landing counts for pressed polymer films.

In contrast to the nylon 6,6 control, the graphene-loaded perforated films did not reduce landings or bites. Unlike the fabric interstices that we were trying to mimic, the perforations in the films had no obstructions (i.e., entangled fibers or loose yarns) to the mosquito proboscis and were much larger in size than what is seen with military uniform fabrics. As such, no conclusions are being drawn from this data.

Small-scale, in-house fiber extrusion was performed utilizing an Xplore HT-15 Microcompounder. In addition to its main function as a compounder, the Xplore Fiber Line provides the ability to collect the extruded material directly as a fiber “strand,” thicker than a traditional fiber but providing the opportunity to knit the strand for analysis as a thick fabric rather than a film. By diluting the 10%wt EGr masterbatch using the Xplore Fiber Line, fiber “strands” were extruded and collected at experimental loading levels. Figure 2 shows fiber strands at 10%, 8%, 5% and 3%wt graphene loading levels. Unfortunately the fiber strands were too brittle and stiff to knit into sleeves for testing.

Figure 2: Nylon 6,6 fiber strands containing (10%, 3%, 5% and 8%wt respectively) graphene loadings.

The first fiber extrusion trial at the TTC was unsuccessful due to pressure issues on the equipment. The graphene masterbatches and control polymer were very viscous and the run was terminated. It was determined that the nylon 6,6 control polymer that was used on its own, and also in the masterbatches, was not a fiber grade polymer. A new 100% nylon 6,6 fiber grade polymer was purchased and used for compounding in the remaining trials.

Although there was some difficulty with viscosity, the second fiber extrusion trial successfully yielded a 100% nylon 6,6 control multifilament fiber and a 1%wt EGr in nylon 6,6 multifilament fiber. The 10%wt masterbatch was let down to 1%wt with the new fiber grade nylon 6,6 which had a lower viscosity and extruded well. The resulting fiber was used to knit swatches of fabric on a circular sock knitter with a diameter of approximately 7.62 cm.

Like the perforated films, the graphene-loaded knit sleeve did not perform well with the control sleeve, taking fewer bites and landings. Although this data is not what was hoped for, it was anticipated based on the data collected for the pressed polymer films in which data indicated that landings were not reduced until the 5%wt graphene range. Further extrusion trials with higher graphene loadings have been unsuccessful due to the high viscosity of the nylon 6,6 when loaded with the graphene. An alternate polymer for extrusion is being explored for additional work.

3.2 Print

All three paste formulations afforded a significant reduction in the bites on the full coverage samples, with the Global Graphene Group (GGG) paste offering the best reduction. In terms of landings, the results are less clear. The Dycotec paste did not produce a reduction in landings as compared to the control. Both the in-house and Global Graphene Group pastes resulted in reductions in landings, with the Global Graphene Group paste having the highest reduction. Results are shown in Figure 3.

The Dycotec paste was easy to use and deposited consistently in thin, uniform traces, while the Global Graphene Group paste demonstrated significant printability issues for intricate patterns that rendered its use to be prohibitive. The Global Graphene Group paste also printed as a stiff barrier as opposed to the thin, more textile-friendly deposition of the Dycotec paste. The in-house paste deposited in a thin, even layer, however, its consistency issues made it challenging to utilize. Additionally, the ethylene glycol component used in the paste wicks significantly into the fabric, discoloring the region surrounding the print. The Dycotec paste did exhibit some wicking through the textile, and the printed region can be faintly seen on the reverse side of the textile, however discoloration surrounding the print did not occur. As such, the Dycotec paste was selected moving forward for patterns and loading levels.

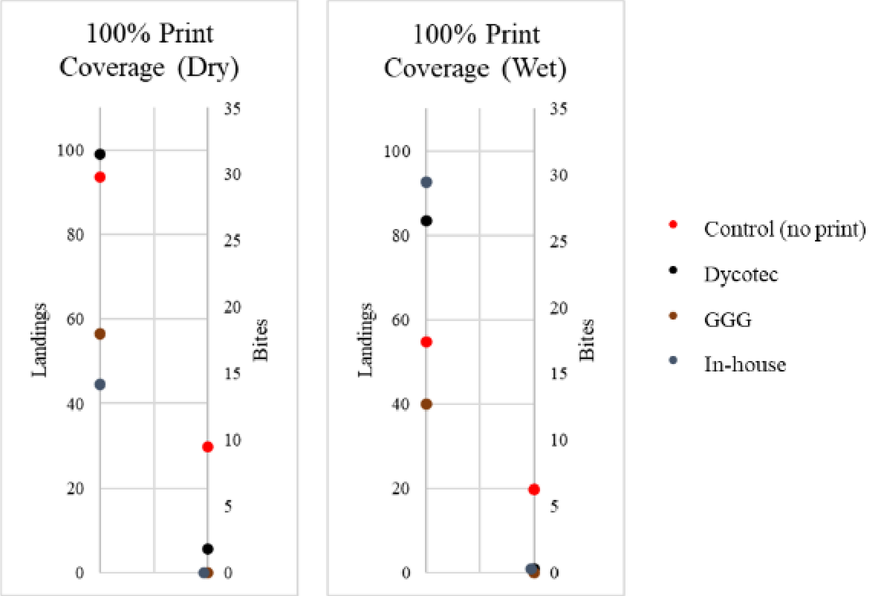


Figure 3: Mosquito landing and bite counts for solid (100% Coverage) printed samples.

3.2.1 Printed Patterns

Four different patterns (dot, grid, lines, and hexagon) with varying areas of fabric coverage were evaluated using the Dycotec paste. The graphene properties of the Dycotec paste are shown in Table 1. Images of each pattern type at 50% coverage are shown in Figure 4.

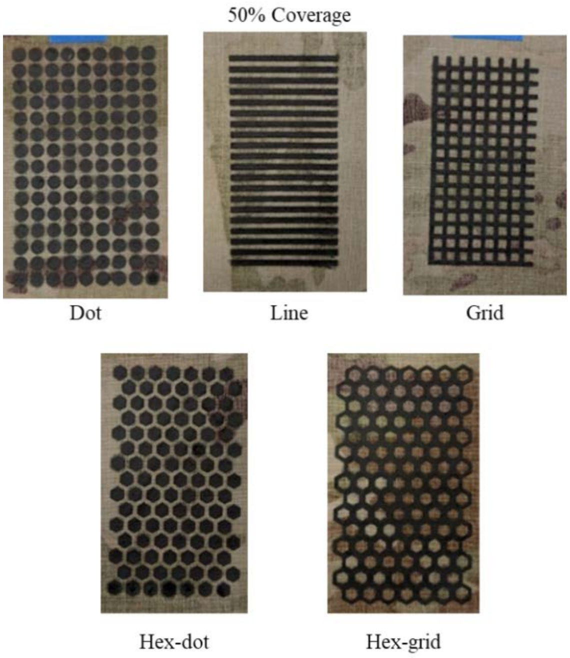


Figure 4: Images of various prints at 50% coverage area.

3.2.1.1 Line Pattern

A variety of line widths and percentage coverages were printed onto the back of the fabrics. Figure 5 shows images of the control swatch and a graphene (Dyco 2500 50%) at one minute and forty-five seconds (1:45) into the three-minute test. The images show an observed difference in landings while the data in Figure 6 shows that the printed samples demonstrated reduced landings and bites. In general, the thinner line width resulted in worse landing performance, however, data for bites was less varied.

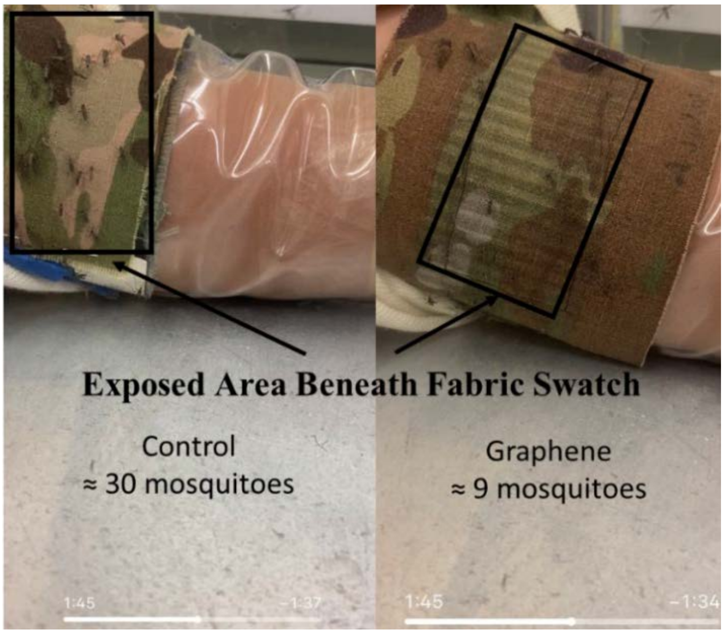


Figure 5: Screenshots of videos taken during testing. The control swatch has no print while the graphene swatch is a line print with 50% coverage area.

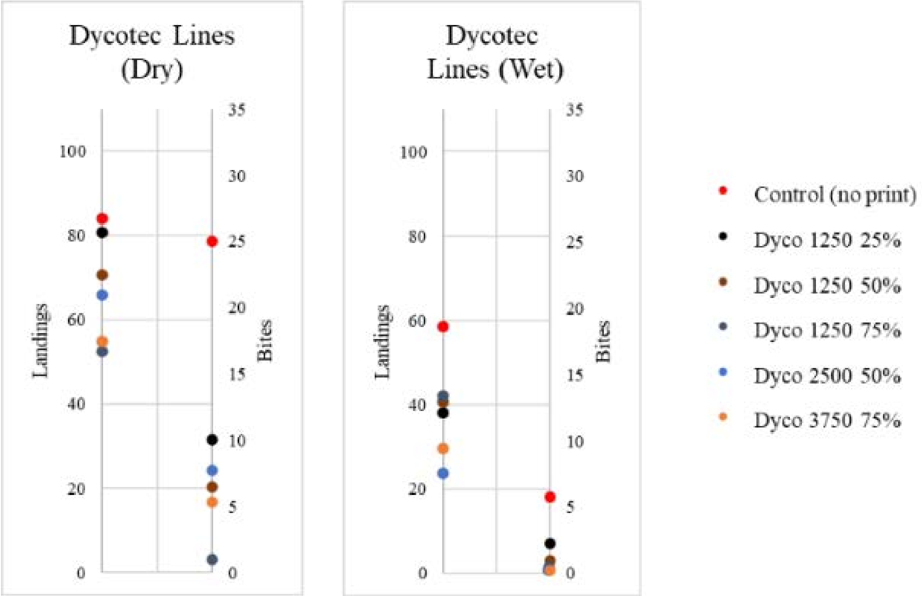


Figure 6: Mosquito landing and bite counts for line patterned samples.

3.2.1.2 Dot Pattern

Various dot sizes and coverage areas were printed onto the back of the fabric. All dot patterned samples demonstrated reduced landings and bites over the control. Data is shown in Figure 7. Increased print coverage did not significantly affect the landings; however, it did reduce the bites slightly. Additionally, the width does not seem to have significantly varied the bite performance as the values are very tightly clustered.

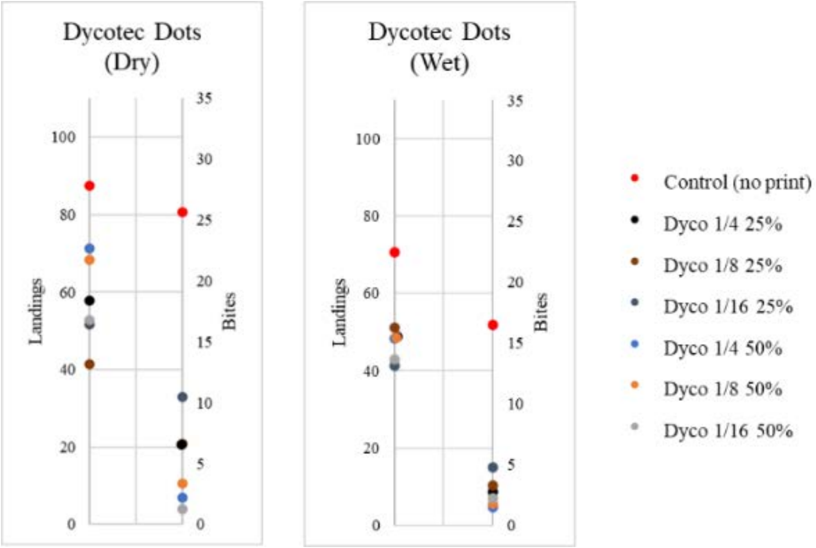


Figure 7: Mosquito landing and bite counts for dot patterned samples.

3.2.1.3 Grid Pattern

As shown in Figure 8, two of the dry grid pattern samples showed similar landing counts to the control, while the remaining samples showed a reduction in landings. All the samples demonstrated a reduction in bites. Generally, increased print coverage improved both landings and bites. Additionally, reducing the feature size generally improved bites counts, but did not strongly affect landings.

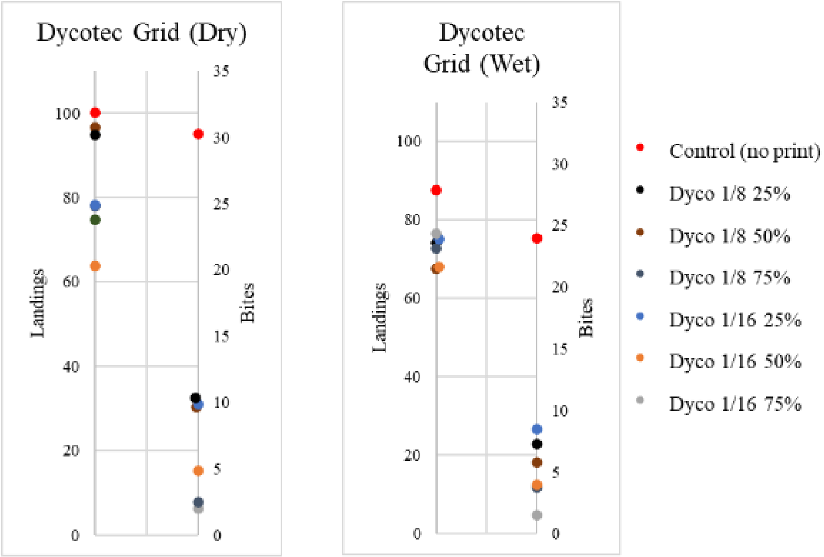


Figure 8: Mosquito landing and bite counts for grid patterned samples.

3.2.1.4 Hexagon Pattern

Two different hexagon patterns with 50% fabric surface coverage were printed. One pattern consisted of an open hexagon grid while the other pattern was with hexagon shaped dots. Figure 9 shows that both patterns showed a noticeable reduction in bites and a slight reduction in landings. While percentage coverage was the same, both pattern types (grid versus dot) performed similarly.

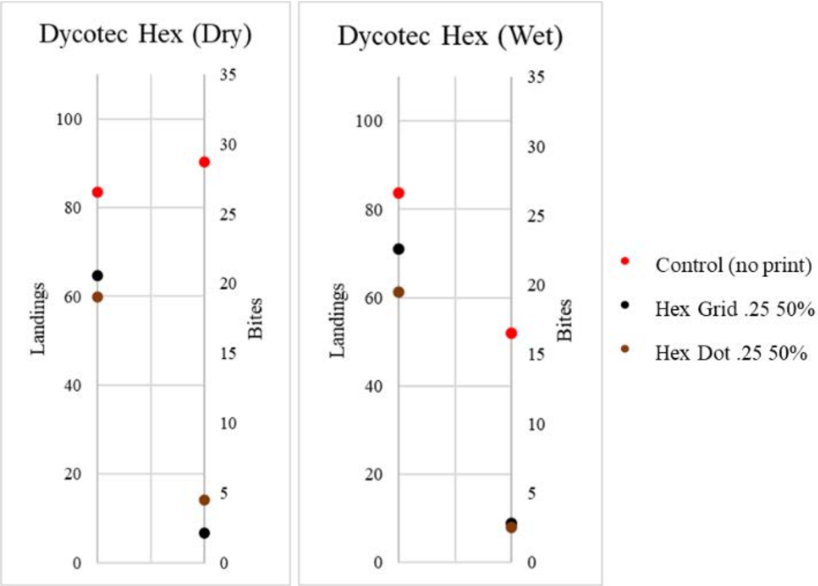
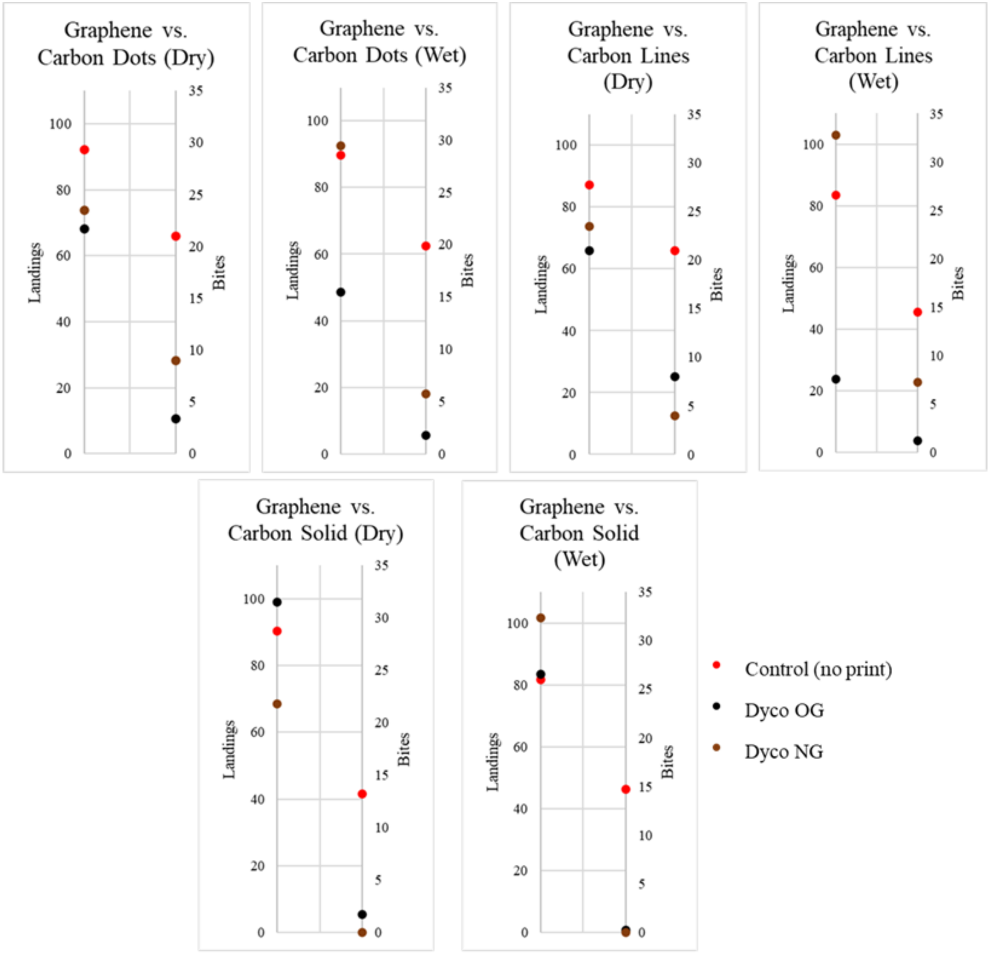


Figure 9: Mosquito landing and bite counts for hexagon patterned samples.

3.2.2 Graphene vs. Carbon Paste

To verify if sample performance was related to another component in the paste or the presence of the paste, as opposed to the graphene specifically, full coverage and limited patterned samples were produced using a custom Dycotec paste modified to exclude the ~10% graphene present in the commercially available paste discussed above. The commercially available paste is composed of 50% a “Blended Filler Mixture,” which includes the graphene component and a balance of carbon black. The custom paste was identical to its counterpart, except the graphene was substituted with carbon black.

The carbon-only paste performed similarly to the graphene paste swatches in terms of landings on the patterned dry samples, with a more noticeable difference in landings on the wet samples. Both pastes performed similarly for bites. It is unclear if it is the presence of graphene or the presence of particulates are responsible for the improved results on most swatches as compared to the control. Results are shown in Figure 10.



Graphene vs. Carbon Dots (Dry)

Graphene vs. Carbon Dots (Wet)

Graphene vs. Carbon Lines (Dry)

Graphene vs. Carbon Lines (Wet)

Graphene vs. Carbon Solid (Dry)

Graphene vs. Carbon Solid (Wet)

Dycotec NG is 0% graphene with carbon filler
Dycotec OG is 10% graphene with carbon filler

Figure 10: Mosquito landing and bite counts for graphene paste vs. carbon-only paste.

3.2.3 Graphene Loading Levels

Print pastes with a variety of graphene loading levels were procured and syringe printed onto the back of the fabric. For these custom pastes, instead of using the “Blended Carbon Filler” used in the commercially available paste a non-carbon filler, titanium dioxide was used. The intent of this filler was to: 1) further demonstrate that the presence of the graphene was producing results; and 2) attempt to lighten the color of the materials. With a carbon black filler for the balance, even a low graphene-loaded sample would appear very dark in color. With titanium dioxide filler, the lower graphene-loaded samples appear grey, which leads to less of an influence on the color of the textile. The paste descriptions to include the original graphene and carbon-only pastes are described in Table 2.

Table 2: Graphene print paste formulations and loading levels.

Paste ID	Balance/Filler	%wt Graphene
Dycotec OG	Carbon	10%
Dycotec NG	Carbon	0%
Solid 0	Titanium Dioxide	0%
Solid 4.6	Titanium Dioxide	5%
Solid G	Titanium Dioxide	10%
Solid 20	Titanium Dioxide	20%
Solid 36	Titanium Dioxide	40%

As shown in Figure 11, all of the pastes with titanium dioxide filler showed a reduction in bites and landings despite graphene percentage (including the 0%). There is too little data to determine if the higher efficacy is due to the presence of titanium dioxide or simply because the coverage of those print pastes was better than that of the OG and NG pastes.

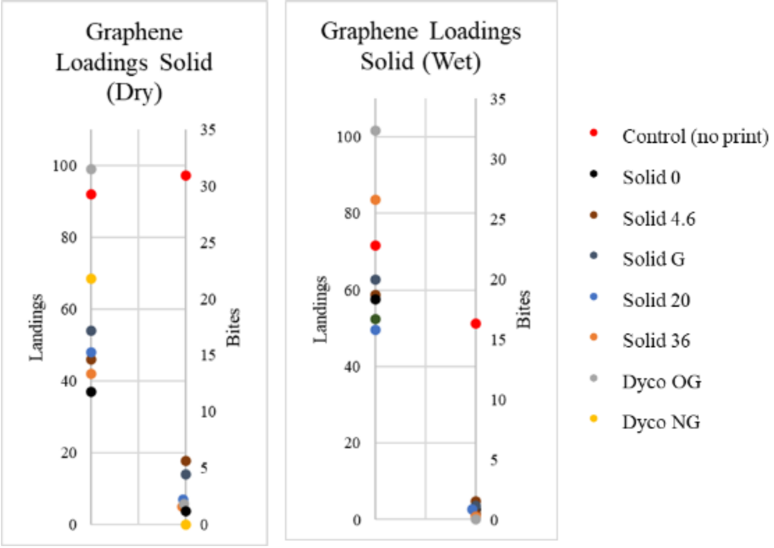


Figure 11: Mosquito landing and bite counts for various graphene loading levels.

4.0 PATH FORWARD

4.1 Polymer Extrusion

Incorporation of solid additives, such as graphene, into textile fibers is not a trivial process. A balance needs to be made between the fiber structure and amount of additive to extrude the blend into a fiber while achieving the desired properties. As discovered in the trials to date, the grade and type of the polymer pellet does influence the extrusion properties once compounded with the graphene. For the most recent trial, the 10%wt EGr masterbatch was compounded with the fiber grade nylon to 3%wt loading level. The compounded polymer was brittle and difficult to extrude. As such, new graphene polymer masterbatches will be procured from the Global Graphene Group using a new fiber grade nylon 6. Masterbatches at 3%wt and 5%wt graphene will be procured for extrusion in-house and at the TTC at Gaston College.

Additional extrusion trials on the new masterbatches will be run to determine if an optimal graphene/polymer ratio for successful extrusion into usable fiber can be found. If successful, we will proceed into further processing into fabrics for efficacy testing. Fibers of approximately 2-denier per filament, the most common filament size associated with currently fielded garments, will be extruded at the various loading levels to enable the understanding of the relationship between the amount of graphene and efficacy performance. Optimal fiber extrusion processing conditions, such as zone temperatures, extrusion speed and draw ratios, will also be determined at this stage. A recently procured Xplore Microcompounder accessory, the multifilament die, will be utilized moving forward to create fibers that are more representative of larger scale extrusion processes capable of producing fiber with lower denier, instead of producing a thick monofilament "strand."

Fiber melt extrusion trials with the compounded materials will be performed starting with the lowest loading level. Once the processing conditions are stable and yield a successful spinnable fiber, the next loading level will be attempted. If yarns are successfully extruded, a combination of knitting and weaving will be utilized to produce fabrics from the fibers to be made into sleeves for efficacy testing. Lessons learned from fiber extrusion, fabric formation and the efficacy results from the samples will aid in further down-selection of polymer / graphene combinations. At the fiber and yarn stage, the possibilities for fiber blends and fabric types are endless. The goal is to produce a variety of yarn and fabric types that can be used as baselines for future development into a fabric that can be used in military uniforms. The development processes for a fabric from start to finish are complex and the production process is long consisting of multiple steps. Fiber extrusion, yarn production, fabric formation and dye / printability will be explored in these outyears.

4.2 Printing

Due to the high cost of print screens, syringe printing continues to be utilized as needed. Mass loading tuned to maximize graphene content without sacrificing printability / durability will continue to be evaluated by printing various graphene loading levels and patterns on the back of current military fabric swatches to determine the effectiveness. Additionally, the team will begin to investigate pastes using graphene oxide. Graphene pastes to date utilized a multi-layer graphene. Pastes will be obtained with graphene oxide using both titanium dioxide and carbon fillers to compare against existing data. Data from this testing will be used to down-select the most promising graphene mass loading levels for laboratory screen scale-up. Work will focus tuning and maximizing graphene content without sacrificing printability / durability.

Based on the printed pattern performance during mosquito testing, four patterns, in addition to a full coverage solid design, were selected for laboratory screen fabrication and procurement. All patterned designs selected offer 50% coverage area to ensure comparison of samples across the patterns. These patterns will also be used with the new set of variably loaded graphene pastes.

Screen printing trials will be completed to optimize print paste viscosity with variably loaded graphene pastes using the full coverage screen. Once viscosity is optimized, formulations will be screen printed for the selected patterns for full arm-in-cage efficacy testing to evaluate the effect of increasing or decreasing the graphene content on performance. The lab samples will also be evaluated for print resolution and further down-selection will take place if necessary.

Because laboratory screen sized samples may not translate to larger scale production and are not large enough for physical testing, larger scale screen printing is required. A capable industry partner will be identified and utilized for low-level scale-up of this effort. Down-selected patterns and print paste formulations will be provided, and full-scale screens procured for the industry partner to print the graphene onto a minimum of 2.5 metres of full-width fabric for further efficacy and physical testing evaluation.

4.3 Testing

The team will continue to collect efficacy data on the small-scale samples to enable further down-selection if necessary.

To date, testing has been completed using a modified version of the USDA MED Test. This modified method will continue to be used for smaller samples, as opposed to the standard full sleeves, to count landings more easily. Specimens will be tested against lab-reared / disease-free *Aedes aegypti* and *Anopheles albimanus* mosquitoes as their host-seeking and biting behaviors are different, and they both are important disease carrying vectors of interest. Bite counts will be taken, while host-seeking behavior will be demonstrated by taking landing counts on the test materials. Data for both mosquito species will be pooled and presented as the average number of landings or bites within the 3-minute test window.

While the mechanism by which graphene prevents biting and landings is still unknown, it is likely multiple mechanisms are involved. Graphene-treated fabrics may act as a physical barrier to mosquito feeding and/or interfere with the potential of mosquitoes to host-seek. Mosquito host-seeking behavior is complex and multi-faceted and includes the detection of host heat, volatiles, and CO_2. Previous groups have suggested that graphene layers are capable of preventing the volatilization of select host odorants, thus potentially preventing mosquitoes from properly sensing their hosts [3]. It is also possible that graphene-treated fabrics affect the thermal profile of the wearer, thus confusing host-seeking mosquitoes or diminishing their desire to feed on the wearer. The aim is to evaluate the potential mechanism(s) that prevent mosquito biting in future bioassays as these studies will be essential to creating future barriers / fabrics that reduce mosquito landing / biting. These studies will assess changes in the thermal profiles of wearers (using forward looking infra-red cameras), changes in host volatile profiles (using analytical chemistry techniques [e.g., gas chromatography-mass spectroscopy]), and even assessing the potential of graphene to produce changes in the neurophysiology of mosquito antennae and tarsi via electrophysiological recordings.

Once larger swatches are produced using screen printing, full arm-in-cage testing in accordance with MIL-PRF-32659 will also be used to calculate the percent bite protection on larger size swatches. This method calculates the percentage reduction in bites (blood-fed mosquito counts) between the “treated” specimen and control using Abbott’s formula [4]. Efficacy data for the two mosquito species will be pooled and analyzed to determine the effect of the graphene technologies on bite protection. Success will be demonstrated by a reduction in bites through the sample materials. Due to the nature of the full arm-in-cage method, it will be difficult to take landing counts, however, if possible, it will be noted if landings appear to be reduced.

In addition to efficacy testing, physical testing will be conducted on large enough samples to determine the effects of the graphene on the properties of the materials to include durability and shade. Degradation of the textiles will be determined by conducing physical testing against known uniform requirements; with the durability of the graphene print being tested via laundering.

4.0 CONCLUSIONS

The pressed polymer films at varying graphene loadings showed a positive initial trend of fewer mosquito landings as compared to the control films. Thickness of the films may be a factor in landing counts, however, even the films that were thinner than the control showed fewer landings. Results of thin films with perforations and of the knit sleeve were not as promising, but the low level of graphene does warrant an additional look at higher loading levels. Unfortunately, issues with the selected polymer for the masterbatch resulted in difficulty extruding usable fibers. Work will continue to look at higher loading levels of graphene in an appropriate fiber grade nylon 6 polymer to determine if extrusion could continue to be a potential option or if the higher graphene loading levels result in too high viscosity for successful extrusion. Continuation of polymer processing of graphene in nylon will be dependent upon the results of the upcoming trials.

Work conducted to evaluate printing graphene on the technical back of the textiles proved more successful. Even with the small sample sets, the mosquito testing supported a conclusion that the printed graphene reduced landings and bites. These positive results warrant additional study to include determining the optimal graphene loading levels in the print pastes and optimizing coverage area without degrading other aspects of the material. This work is currently in process. Positive data also indicates that the project can move to laboratory scale-up, which will be performed through producing larger samples through a more scalable manufacturing method, screen printing. Once in-house screen printing provides additional data for pattern selection, even larger samples can be produced out-of-house.

The major technical risk will be to determine if dispersed or patterned graphene can interfere with mosquito host-seeking behavior, and, if so, the level and distribution of graphene that is required. Key factors in transitioning into military uniforms include whether the technology shows an effect on mosquito host-seeking behavior for a reduction in bites without detrimentally affecting the required physical properties of the materials. The metric for success is a reduction in landings and/or a reduction in bites as compared to a control sample. At this stage in development, degradation of material properties is only of slight concern as adjustments are possible with further development and optimization. Back-printing may only bleed-through or affect shade on lighter-weight military fabrics and not the heavier materials such as the Advance Combat Uniform. Additionally, many physical and durability properties can be improved with proper fiber blend, yarn size selection, and weave construction.

Overall, the data indicates that further research is needed and warranted to determine if graphene can be incorporated into textiles in a way that has little to no negative effects on the physical properties of the uniform materials, while minimizing potential exposure to disease carrying vectors. Initial research showed that the samples produced did influence the mosquito behavior, although it is too early to tell if reduced landings and bites were due to the graphene or changes in sample physical factors.

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6.0 REFERENCES

[1] Burnette, W.N., C.H. Hoke, Jr., J. Scovill, K. Clark, J. Abrams, L.W. Kitchen, K. Hanson, T.J. Palys, Vaughn, D.W. Infectious diseases investment decision evaluation algorithm: A quantitative algorithm for prioritization of naturally occurring infectious disease threats to the U.S. Military, Mil. Med. 173: 174-181. 2008.

[2] Agramonte, N.M., Bloomquist, J.R., Bernier, U.R. Pyrethroid resistance alters the blood-feeding behavior in Puerto Rican Aedes aegypti mosquitoes exposed to treated fabric. PLOS Neglected Tropical Diseases. 11(9). 2017.

[3] Castilho, C.J., Li, D., Liu, M., Liu, Y., Gao, H., Robert, H., Hurt, R.H., Mosquito bite prevention through graphene barrier layers. PNAS. 116: 18304-18309. 2019.

[4] Abbott, W.S., A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-264. 1925.

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