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An optimized artificial blood feeding assay to study tick cuticle biology

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ABSTRACT

Ticks, ectoparasitic arachnids, are prominent disease vectors impacting both humans and animals. Their unique blood-feeding phase involves significant abdominal cuticle expansion, sharing certain similarities with insects. However, vital aspects, including the mechanisms of cuticle expansion, changes in cuticular protein composition, chitin synthesis, and cuticle function, remain poorly understood. Given that the cuticle expansion is crucial for complete engorgement of the ticks, addressing these knowledge gaps is essential. Traditional tick research involving live animal hosts has inherent limitations, such as ethical concerns and host response variability. Artificial membrane feeding systems provide an alternative approach, offering controlled experimental conditions and reduced ethical dilemmas. These systems enable precise monitoring of tick attachment, feeding parameters, and pathogen acquisition. Despite the existence of various methodologies for artificial tick-feeding systems, there is a pressing need to enhance their reproducibility and effectiveness. In this context, we introduce an improved tick-feeding system that incorporates adjustments related to factors like humidity, temperature, and blood-feeding duration. These refinements markedly boost tick engorgement rates, presenting a valuable tool for in-depth investigations into tick cuticle biology and facilitating studies on molting. This refined system allows for collecting feeding ticks at specific stages, supporting research on tick cuticle biology, and evaluating chemical agents' efficacy in the engorgement process.

1. Introduction

The arthropod exoskeleton, or the cuticle, is primarily made up of chitin and a complex mixture of proteins (Willis, 2018). It protects soft-bodied insects from dehydration, infectious agents and facilitates essential gas exchange (Merzendorfer and Zimoch, 2003; Muthukrishnan et al., 2020; Zhu et al., 2016). Several studies have used insect model systems, including *Drosophila melanogaster* and *Tribolium castaneum*, to advance our understanding of cuticle development and function (Merzendorfer and Zimoch, 2003; Moussian, 2010; Muthukrishnan et al., 2020; Zhu et al., 2016). These models offer advantages like easily accessible anatomy, rapid life cycles, and genetic manipulability and have contributed significantly to understanding the molecular composition, mechanical characteristics, and functional significance of the cuticle in arthropod molting and metamorphosis. Nonetheless, relying solely on data from model insects may prove inadequate, given the potential differences in cuticle architecture among various arthropod species that cater to specific adaptations.

Ticks are ectoparasitic members of the arachnid family and serve as vectors for many diseases that can be transmitted to humans and animals, thus posing considerable health risks (Bernard et al., 2020; Bobe et al., 2021; Leitner et al., 2015; Rochlin and Toledo, 2020). Throughout their life stages, ticks rely on mammalian hosts to obtain a blood meal, which is crucial for their molting and embryogenesis processes (Kocan et al., 2015). During the blood-feeding phase, ticks substantially expand the surface area of their abdominal cuticle to accommodate a large blood meal, which suggests that ticks may have evolved unique cuticular modifications compared to other arthropods with rigid cuticles (Andersen and Roepstorff, 2005; Starck et al., 2018). Like insects, tick cuticles are composed of intricate layers of chitin, proteins, and lipids (Andersen and Roepstorff, 2005; Lees, 1952). However, a mechanistic understanding of cuticle expansion during blood feeding, changes in cuticular protein composition, changes in rates of chitin synthesis and assembly, and cuticle function remains largely unknown.

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Given the importance of the cuticle as a target of chemical intervention against arthropods, addressing the above knowledge gaps would be crucial for developing innovative strategies to combat tick-borne diseases (Cohen, 1993; Doucet and Retnakaran, 2016; Merzendorfer and Zimoch, 2003). Tick researchers have traditionally relied on feeding models involving live animal hosts, typically mice. While this approach allows the study of natural tick-feeding behavior, pathogen transmission, and host impact, it has inherent limitations, such as ethical concerns, host response variability, inability to obtain precisely timed samples, and significant expenses of conducting such experiments.

In contrast, artificial membrane feeding involves simulating live animal tick feeding using an artificial chamber system. These systems use artificial membranes with controlled environmental conditions to facilitate and monitor tick attachment and feeding. Artificial feeding offers several advantages over live animal models, such as standardized experimental conditions, the ability to manipulate tick feeding parameters, and reduced ethical concerns associated with animal use. Using artificial feeding systems, researchers can precisely monitor factors like tick attachment, feeding duration, as well as the acquisition and transmission of pathogens (Bullard et al., 2016; Fourie et al., 2019; Gonzalez et al., 2021; Koci et al., 2018; Krober and Guerin, 2007a, 2007b; Oliver et al., 2016).

While multiple reports have emerged in recent years outlining methodologies for artificial tick-feeding systems, there remains a need to improve the reproducibility and overall effectiveness of these systems (Bullard et al., 2016; Fourie et al., 2019; Garcia Guizzo et al., 2023; Gonzalez et al., 2021; Koci et al., 2018; Krober and Guerin, 2007a, 2007b; Oliver et al., 2016). In this context, we present an improved tick-feeding system incorporating various adjustments to the existing methodology. We have fine-tuned modifications to factors like humidity, temperature, and the duration of blood feeding, which provides distinct advantages in enhancing the overall engorgement rate of feeding ticks. Our refined feeding system is an invaluable tool for studying cuticle biology in ticks, enabling the collection of feeding ticks at precise feeding stages and facilitating molting studies on fully engorged ticks at specific time intervals. Consequently, this improved feeding system can be further employed for comprehensive investigations into tick cuticle biology and evaluation of the effectiveness of chemical agents on the engorgement process.

2. Material and methods

2.1. Ticks

Ixodes scapularis unfed nymphs were obtained from the Centers for Disease Control and Prevention through the Biodefense and Emerging Infectious Diseases (BEI) resources for performing the artificial blood feeding assays. The fully engorged *Ixodes scapularis* nymphs fed on New Zealand white rabbits were procured from the Oklahoma State University tick rearing facility. Unfed or fully fed nymphs were maintained in glass desiccators with 12-h dark-light cycle at room temperature with >95% relative humidity.

2.2. Silicon membrane preparation

Silicon membranes were prepared as described earlier (Bullard et al., 2016; Koci et al., 2018; Krober and Guerin, 2007a, 2007b; Oliver et al., 2016), with several modifications. Briefly, Tiffen Lens Cleaning Tissue Paper (Fisher Scientific, Catalog # NC9731809) was placed on the smooth side of black poster board (Office Depot, Item #858430) and securely taped in position. Ecoflex Supersoft 0010 Silicon Rubber, consisting of component A and component B (Smooth-On, Easton, PA), was mixed in a 1:1 ratio (5 ml each). Subsequently, 2.5 ml of Hexane (Sigma, 34859-1L) was introduced to the 10 ml silicon mixture to reduce viscosity. The components were thoroughly mixed before applying the silicon mixture onto the lens paper.

The silicon mixture was evenly spread across the lens paper to form a thin silicon rubber film using a silicone squeegee (Fig. 1A). The paper was carefully scraped using the squeegee until no excess silicon remained. The membranes were allowed to solidify for 20 min. Subsequently, they were gently detached from the black poster boards and left on the benchtop overnight before assembling the chambers.

2.3. Tick feeding chamber assembly

Tick-feeding chambers were prepared as described earlier (Bullard et al., 2016; Fourie et al., 2019; Koci et al., 2018; Krober and Guerin, 2007a, 2007b; Kuhnert et al., 1995; Oliver et al., 2016), albeit with several modifications. Briefly, an acrylic plastic tube with a diameter of 1.25 inches (ePlastics, Catalog # ACREXT1.250X1.125) was utilized and 2-inch-long sections were sawed off for tick-feeding chambers. These acrylic tube sections were swiftly heated on a heat plate under a hood to flatten uneven edges. Subsequently, the previously dried silicon membranes were affixed to the tube sections using Loctite® Clear Silicone Sealant (Loctite Products) (Fig. 1A) to form the feeding chambers. Any excess membrane was carefully trimmed off the feeding chambers the following day, and a leak test was conducted. The leak test involves immersing the membrane end of the feeding chamber in a 6-well plate (Fisher Scientific, catalog # 0720080) filled with 70% ethanol (4 ml) for 20 min (Fig. 1B). During this period, the chambers are periodically rotated to inspect for leaks from its cylindrical edges. Chambers that remained intact (without leaks) were then left to dry overnight before being employed for tick feeding.

2.4. Deer hair lipid extraction

The deer hair lipid extraction was performed as described previously with some modifications (Koci et al., 2018). Briefly, 0.25 g of finely chopped deer hair were placed it in a 5 ml screw-cap glass culture tube. Deer hair was obtained from a meat processing plant in Omaha, Nebraska. Subsequently, 5 ml of dichloromethane (Sigma, catalog # 270997-1L) was added to pre-weighed deer hair and the hair gently pushed down into the solution using a glass rod to ensure complete immersion. The deer hair was allowed to steep in dichloromethane for 2 days. We routinely retrieve 5 ml of extract and then further reduce it to a total volume of 0.5 ml by heating the collected extract in a glass beaker on a hot plate at 40 °C in a fume hood. The concentrated deer lipid extract (0.5 ml) was subsequently stored in small glass vials at −20 °C until further use. Deer hair lipid extracts prepared in this manner can be stored for 4 weeks at −20 °C without significant loss of potency.

2.5. Tick-feeding

Before commencing tick feeding, the chambers were treated with deer hair lipid extract. Specifically, 50 μl of deer hair lipid extract was carefully added to the center of the membrane, taking care to prevent seepage into the silicon glue. Once the extract had evaporated, the chambers were inverted and placed in a 45 °C incubator for 20 min to facilitate complete evaporation of dichloromethane.

The dried chambers were immediately utilized to feed ticks. A total of 16 ticks were introduced into each chamber using a fine brush with white bristles, and the chambers were sealed with white foam plugs (VWR, catalog # 60882-189). To each well of a 6-well plate, 4 ml of defibrinated bovine blood (Fisher Scientific, catalog # 50-413-857) supplemented with 10 mM glucose, 1 mM ATP, and an antibiotic-antimycotic cocktail containing 10,000 units/ml of penicillin, 10,000 μg/ml of streptomycin, and 25 mg/ml of amphotericin B was added.

The chambers containing ticks were carefully placed in 4 ml bovine blood being careful to avoid any formation of bubbles at the blood-membrane interface (Fig. 1C). Subsequently, the 6-well plates containing chambers were positioned in a 5 L water bath (VWR, catalog # 76308-834) set at 34.5 °C for the initial 48 h and then at 36.5 °C for the

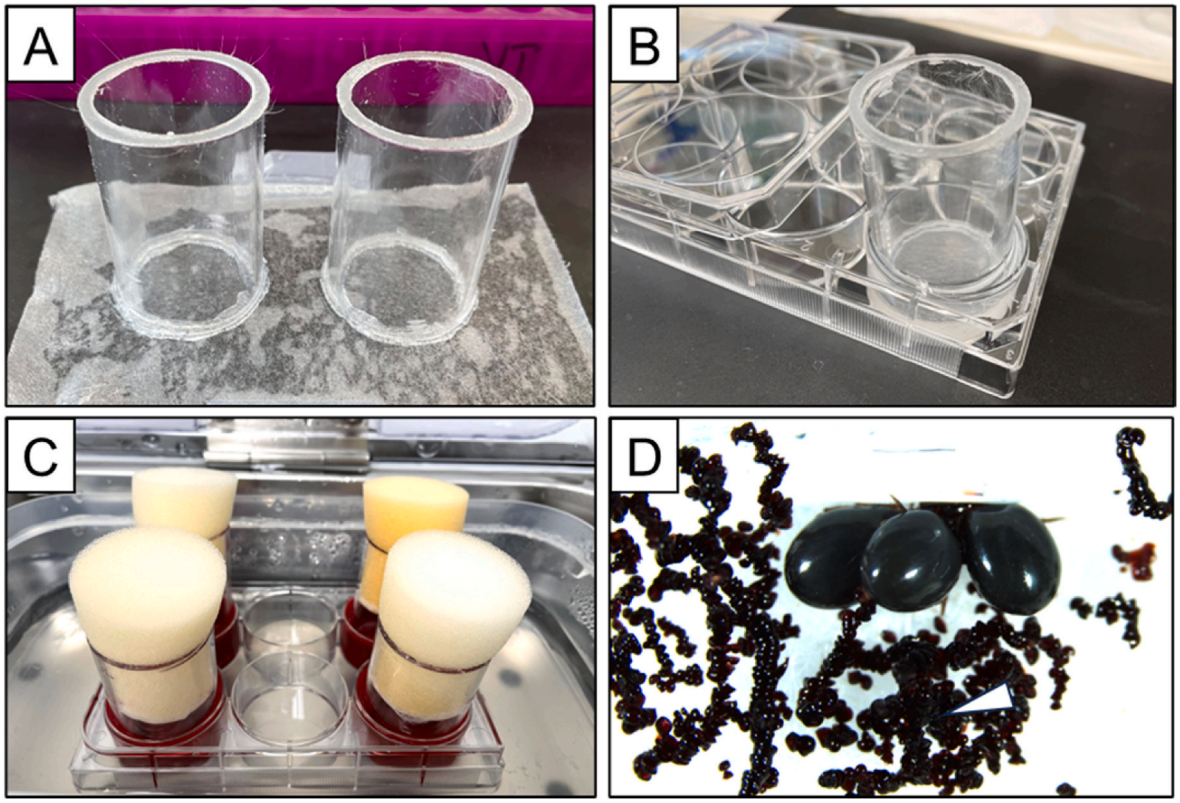

Fig. 1. Assembly of artificial tick feeding chambers. (A) Two-inch acrylic tubes were attached to the silicone membrane using silicone glue. (B) A leak test was performed by submerging the tick feeding chamber in 70% alcohol for 20 min. (C) Assembled tick feeding chambers were placed in a 6-well plate containing 4 ml of defibrinated bovine blood, supplemented with 10 mM glucose, 1 mM ATP, and an antibiotic-antimycotic cocktail containing 10,000 units/ml of penicillin, 10,000 mg/ml of streptomycin, and 25 mg/ml of amphotericin B. A total of 16 ticks were added to each chamber for individual experiments. (D) Ticks attached and engorged on the artificial membrane. Excreta (labeled with a white arrow) indicate a successful feeding process for the ticks on the membrane.

remainder of the feeding process, spanning the next 5 days. Blood changes were performed at 12-h intervals during the first 48 h, during which ice-chilled blood was introduced into the 6-well plates. After 48 h, blood changes were performed every 24 h, with prewarmed blood at 37 °C added to the 6-well plate. During blood changes, the silicon membranes are quickly rinsed with 1X PBS and promptly inserted into new bovine blood wells. This process is completed within a few seconds to maintain continuous tick feeding.

2.6. Tick imaging and scutal index (SI) determination

Ticks collected at various time points during the feeding stages were photographed using the Leica M165 FC Stereomicroscope at 45X zoom. For the determination of the scutal index, selected images were imported into PowerPoint, where the width of the scutum and the length of the alloscutum was measured using the PowerPoint measurement tool. The Scutal Index (SI) was then calculated by taking the ratio of the length of the alloscutum to the width of the scutum.

2.7. Transmission electron microscopy (TEM)

Fully engorged ticks were collected and prepared for transmission electron microscopy (TEM) following a previously established protocol (Chaudhari et al., 2011, 2013) with some minor adjustments. The specimens were initially dipped in propylene oxide for a few seconds, dried with Kim wipe papers and fixed in a solution containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) for 24 h at room temperature while constantly rotating. Samples were then washed three times for 5 min each in 0.1 M sodium cacodylate buffer at room temperature with constant rotation. Subsequently, the samples were post-fixed using a 1% osmium tetroxide solution in 0.1 M sodium cacodylate buffer at room temperature with constant rotation for 1–2 h. Post fixation, samples were washed three times for 5 min each in 0.1 M sodium cacodylate buffer at room temperature with constant rotation. An ascending ethanol gradient was employed to achieve complete sample dehydration, progressing through concentrations of 70%, 80%, 90%, 95%, and three rounds of 100% (each cycle for 30 min). The samples were then infiltrated with a mixture of EMBED 812/Araldite resin (1:1 propylene oxide: resin) for 48 h, followed by vacuum infiltration with 100% resin for 6 h. Subsequently, the specimens were embedded in flat molds, and the resin was solidified by curing in a drying oven at 65 °C for 24 h. After curing, the samples were trimmed, sectioned into silver or gold sections (100 nm), attached to 200 mesh copper grids, and then stained with 1% uranyl acetate and 1% lead citrate. Finally, the samples were imaged using a CM-100 FEI Tecnai G2 Spirit transmission electron microscope.

3. Results

3.1. Optimization of the tick artificial feeding assay

A series of experiments were conducted to optimize the efficiency of tick feeding, focusing on various parameters, including temperature, humidity, and the frequency of blood replacement. Initially, we carried out *in vitro* feeding of *I. scapularis* nymphs in a CO_2 incubator set at a temperature of 37 °C, 60% humidity and CO_2 levels of 5%. Over a 10-day incubation period, fewer than 20% of the nymphs attached to the membrane and less than 2% achieved complete engorgement (Fig. 2A). Even among the attached ticks, only partial engorgement was observed.

To assess the effect of humidity within the feeding chambers, we

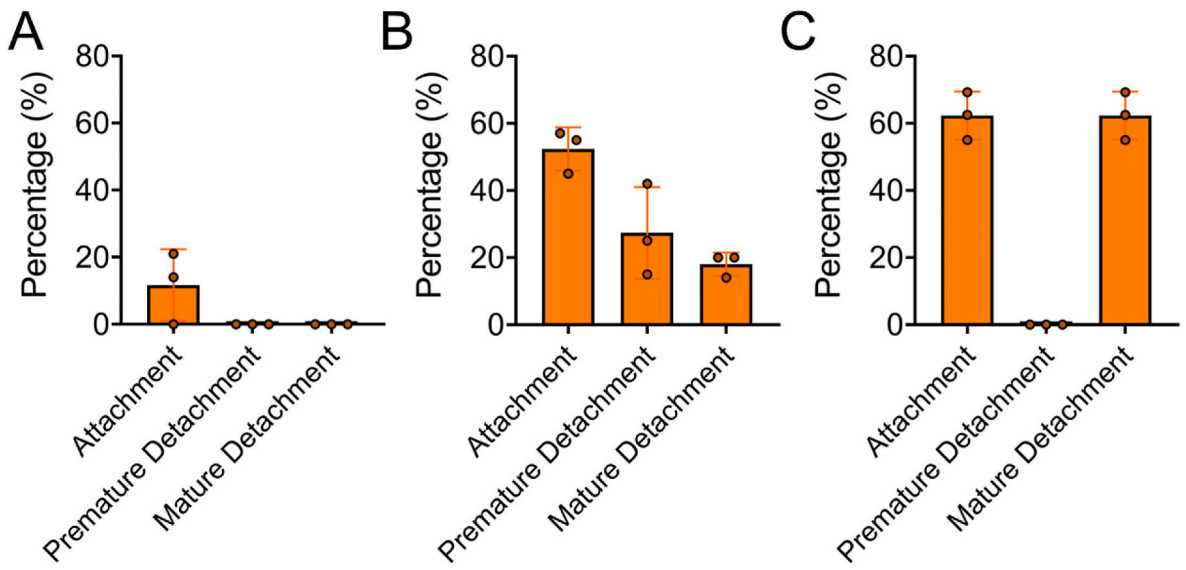

Fig. 2. Artificial feeding assay for *I. scapularis* Nymphs. Ticks were fed on artificial membranes in (A) a CO_2 incubator set at a temperature of 37 °C with CO_2 levels at 5% and 60% humidity, (B) a 37 °C water bath (>95% humidity) with the addition of prewarmed blood (37 °C) every 12 h, and (C) a water bath initially set at 34.5 °C for the first 48 h with the addition of chilled blood, followed by an adjustment to 36.5 °C with the addition of warm blood for the subsequent 5-day period (>95% humidity). Attachment: Initially attached ticks to the membrane; Premature detachment: Partially fed ticks detached prematurely; Mature detachment: Fully engorged ticks detached after completion of feeding. Indicate statistics.

conducted a comparable experiment using a water bath set at a humidity level exceeding 95%. We placed a six-well plate containing the tick-feeding chambers in a 37 °C water bath and added prewarmed blood (37 °C) every 12 h. This led to an increased attachment rate, reaching approximately 50%. Although we observed a higher rate of complete nymphal engorgement, there was still a relatively high rate of premature detachment among partially fed nymphs during the 7-day incubation period (Fig. 2B).

To further enhance the attachment rate and minimize the premature detachment of partially fed nymphs from the membranes, we adjusted the water bath temperature, blood temperature and the frequency of blood changes during the experiment. The introduction of ice-chilled

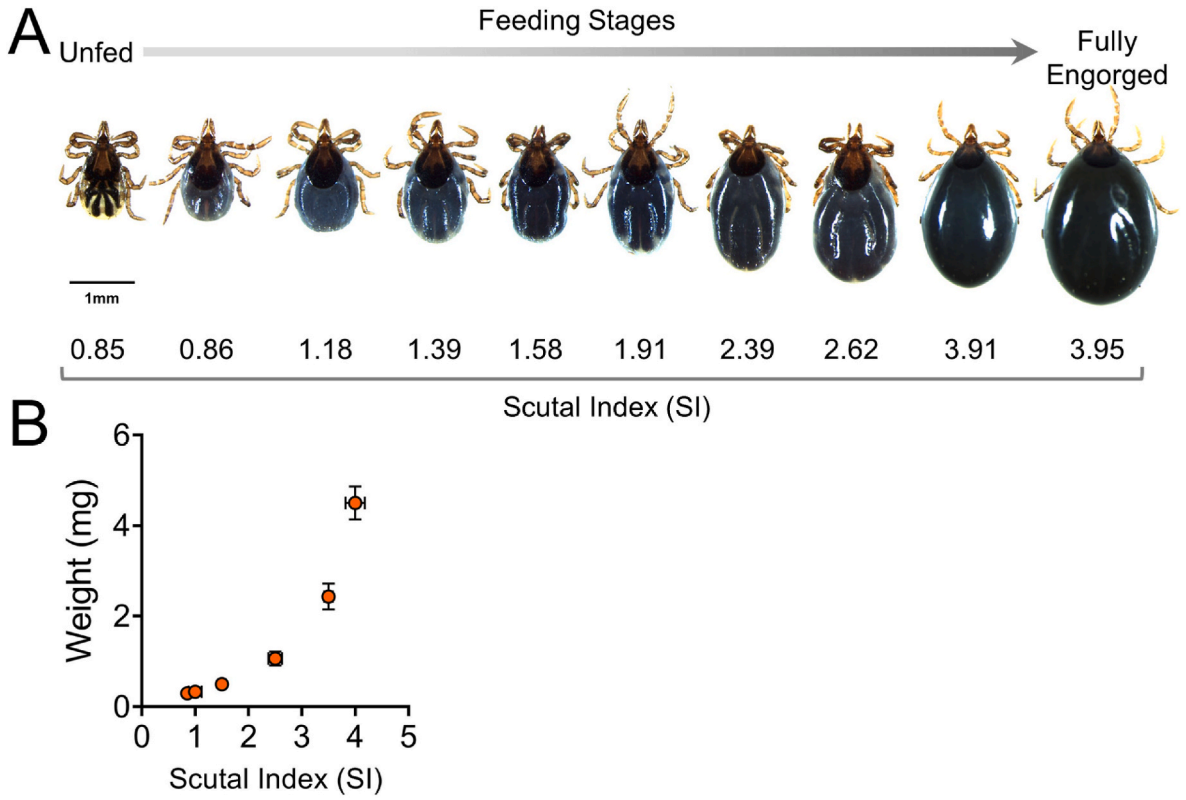

Fig. 3. Scutal Index of *I. scapularis* Nymphs Feeding on Artificial Membranes. (A) Ticks were collected to determine the gradual increase in their scutal index during the feeding process. The scutal index was determined by measuring the ratio between the alloscutum and the scutum, as described in materials and methods. (B) A direct comparison between tick weights and the SI at various feeding stages (n = 3, mean ± SD).

blood for the first 48 h, with blood changes occurring every 12 h and the water bath temperature set to 34.5 °C led to an increased attachment rate of >60%. Following 48 h, warm blood changes (maintained at 37 °C) were initiated every 24 h and the water bath temperature was raised to 36.5 °C, which completely eliminated tick premature detachment. This optimized method, which included maintaining humidity above 95%, controlling the water bath and blood temperatures, ensured the complete engorgement of nearly all initially attached nymphs (Figs. 1D and 2C).

3.2. Tick collection at different scutal indices

To accurately gauge the extent of feeding on artificial membranes, we collected nymphal *I. scapularis* ticks at various phases of their feeding process and determined their scutal index (SI). The SI serves as a widely recognized measure of tick feeding and is calculated as the ratio of the length of the tick alloscutum and the width of the scutum (Meiners et al., 2006). The nymphal ticks were gently removed from the membrane using blunt forceps and inspected using a stereomicroscope. The abdominal length of the ticks was measured with an eyepiece reticle ruler. From the initial attachment to the fully fed stage, we observed a gradual increase in tick scutal index (ranging from 0.85 to 3.95) over 5 days (Fig. 3A), indicating successful tick feeding on the artificial feeding membranes. Further, to investigate the correlation between the degree of feeding and the SI, we conducted a direct comparison between tick weights and the SI at various feeding stages. Our analysis indicates that the changes in the tick SI are exponentially proportional to their weights (Fig. 3B).

3.3. Assessing efficiency of the tick feeding process

The efficiency of tick feeding was assessed based on the molting rate of nymphs to adults. Fully engorged nymphs that detached from the membrane in the feeding chamber were categorized into two groups based on their scutal index (SI): group 1, with SI ranging from 3.0 to 3.5, and group 2, with SI ranging from 3.5 to 4.6 (Fig. 4A). Consistent with differences in SI, the measured weights of the group 1 ticks averaged 2.46 mg, while group 2 ticks were an average of 4.42 mg (Fig. 4B).

Upon further incubation of ticks from both groups in a desiccator at room temperature, maintained at a relative humidity of greater than 95%, ticks underwent molting. Approximately 35 days after complete engorgement, ticks in group 1 transformed into adult males, whereas

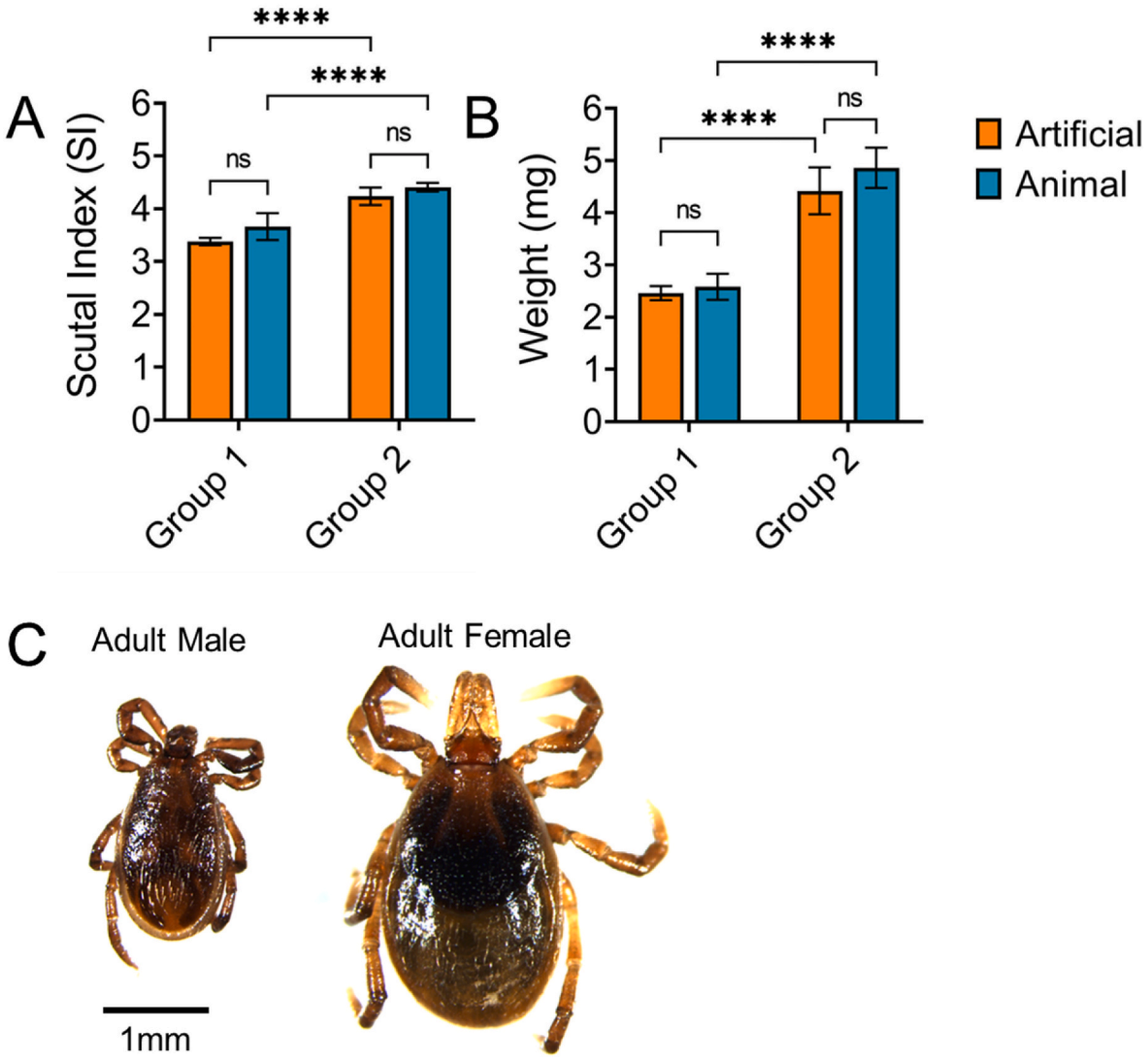

Fig. 4. Assessing the Success of the Artificial Feeding Process. (A) Fully engorged ticks collected from New Zealand white rabbits or artificial chambers were divided into two groups according to their scutal index (SI): Group 1, SI 3.0–3.5, and Group 2, SI 3.51–4.6 (n = 5). The final weights (B) and molting success (C) of both these groups were assessed. Ticks with lower weights in group 1 molted into adult males, whereas those with greater weights in group 2 developed into female adults. Two-way ANOVA with Tukey's multiple comparisons test; ****P ≤ 0.0001.

ticks in group 2 successfully molted into adult females (Fig. 4C). All fully engorged ticks incubated in this manner successfully molted.

Further, we performed a comparative analysis between animal fed versus artificial chamber fed nymphs. Our comparative data shows no significant difference in tick weights, scutal index, and molting time (Fig. 4A and B).

3.4. Cuticle architecture of fully engorged tick

To further evaluate the efficacy of the artificial feeding system as a tool for investigating tick cuticle biology, we conducted an examination of the ultrastructure of fully engorged nymphal cuticles using transmission electron microscopy (TEM). Previous studies of Ixodid tick cuticles have documented alterations in the architecture of the alloscutal cuticle during the blood-feeding process (Beadel, 1974; Flynn and Kaufman, 2011, 2015; Hackman, 1975; Lees, 1952; Starck et al., 2018). Fully engorged tick cuticles have been shown to exhibit characteristics such as the loss of cuticular folds in the epicuticle, an increase in the number of pore canals within the exocuticle, and an enlargement of the endocuticle (Beadel, 1974; Flynn and Kaufman, 2011, 2015; Hackman, 1975; Starck et al., 2018).

Our findings within the artificial feeding system revealed that the architecture of cuticles from fully engorged ticks exhibited close similarities to those previously observed (Hackman, 1975; Starck et al., 2018). The TEM analysis of the fully engorged *I. scapularis* nymphs on the artificial membrane displayed the presence of three layers: the outermost envelope, the middle epicuticle, and the innermost procuticle (Fig. 5A). Notably, the procuticle was further subdivided into two morphologically distinct layers, the exocuticle and endocuticle. The exocuticle exhibited multiple pore canals (Fig. 5B) as described earlier, while the endocuticle displayed a laminar architecture (Fig. 5C) (Hackman, 1975; Starck et al., 2018), similar to what was observed in model insect species such as *Tribolium castaneum* and *Drosophila melanogaster* (Noh et al., 2014, 2017; Tajiri et al., 2017; Wolfgang et al., 1986). Furthermore, our comparison of *I. scapularis* nymphs fed on blood in artificial feeding chambers to those fed on New Zealand white rabbits, suggest very similar ultrastructural features (Fig. 5D–F). This suggests that the artificial model system serves as a viable alternative to tick feeding on animals, allowing for experiments to be conducted in a more controlled environment with greater precision.

4. Discussion

In the past, *in vitro* membrane feeding systems have been proposed as a method for tick feeding. While these systems have shown some success in feeding ticks without the reliance on animal hosts, there were clear deficiencies due to a lack of consistency in tick feeding and engorgement rates between test chambers. In our study, we successfully optimized multiple aspects of existing artificial tick feeding methodology to improve the overall efficiency of tick feeding and assessed its suitability for studying the tick cuticle physiology under tightly controlled conditions.

Modifying parameters such as temperature, humidity, and the frequency of blood replacement significantly improved the overall tick attachment rate and feeding efficiency. Notably, maintaining humidity levels close to 95% was crucial, as a lack of humidity severely hindered the tick attachment rate in the CO_2 incubator, reducing it to below 20%. However, when these same experiments were performed in a water bath, the attachment rate increased to over 50%. This observation agrees with previous findings (Oliver et al., 2016), which described humidity as a critical factor for achieving optimal attachment in feeding chambers.

Furthermore, we noted that a delicate balance between humidity and temperature was necessary for enhancing the attachment rate within artificial feeding chambers. A mere decrease in the water bath temperature from 36.5 °C to 34.5 °C for the first 48 h of the 7-day feeding had a

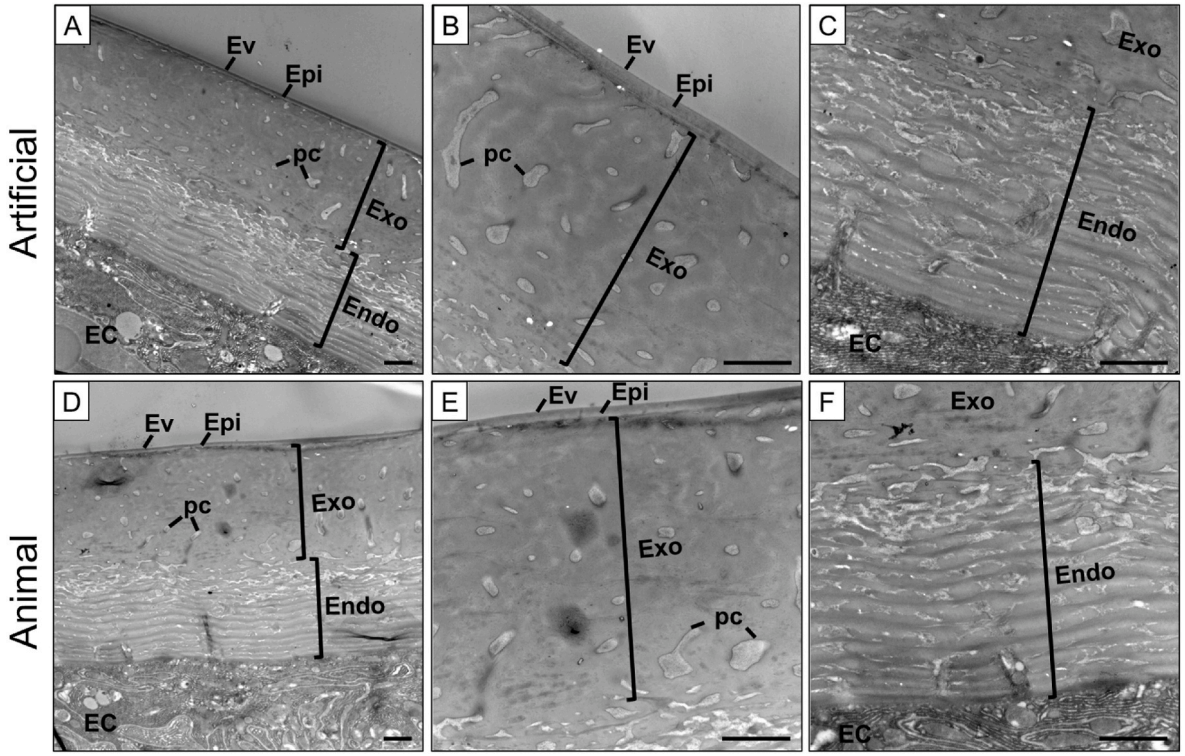

Fig. 5. Ultrastructure of fully engorged nymphal alloscutal cuticle. A TEM-based ultrastructural comparison of the dorsal alloscutal cuticle in fully engorged *Ixodes scapularis* nymphs obtained from artificial feeding chambers (A–C) and New Zealand white rabbits (D–F). Panels (B, E) and (C, F) respectively present magnified images of the exocuticle and endocuticle. Ev, envelope; Epi, epicuticle; Exo, exocuticle; Endo, Endocuticle; PC, pore canal; EC, epithelial cell. Scale bars, 2 μm.

substantial impact, further increasing the attachment rate to over 60%. We also found that using ice-chilled bovine blood for the first 48 h initially helped keep the ticks on the membrane surface for extended periods, leading to increased attachment rates. Conversely, when warm blood was used for the first 48 h, the attachment rates dropped significantly (data not shown).

One aspect that has not been thoroughly addressed in previously developed artificial tick feeding systems is how to prevent premature detachment of partially fed ticks during the artificial membrane feeding process. We encountered a challenge in the premature detachment of ticks from the membranes before achieving complete engorgement. Our findings revealed that the frequency of blood changes, once the ticks were attached to the membrane, is critical in determining the engorgement rate and preventing premature detachment. When blood changes were performed at 12 h intervals throughout the 7-day feeding, the ticks detached prematurely at varying scutal indices before reaching a fully engorged state. In contrast, switching to a 24 h blood change interval allowed the ticks to achieve complete engorgement without detaching prematurely.

Our modifications enhance the tick artificial feeding system, making it a more valuable resource than animal model systems. This system is particularly beneficial for studies focused on developing chemical intervention strategies against molecular targets in ticks and gaining insights into the inhibitory mechanisms of these interventions. These modifications enable precise control over the feeding of ticks with compounds of interest and offer the flexibility to adjust experimental parameters with ease. Also, the optimized feeding system will prove invaluable for studying tick cuticle biology and the molting process. Within these specialized feeding chambers, nymphal *I. scapularis* ticks undergo a complete feeding cycle, attaching to the membrane and feeding until engorged, typically lasting five days. Moreover, we have confirmed the full engorgement of these nymphs within our feeding chambers through a molting test and weight determination. Following complete engorgement, the *I. scapularis* nymphs successfully molt into adults after approximately 35 days of incubation within the desiccator. The adult male and female sex ratios we obtained using the optimized tick feeding chambers closely align with previously reported correlations between the weights of fully engorged ticks and their respective sexes; that is ticks with greater weight following engorgement develop into females, while those with lower weights become males (Rowley and Hu, 2000).

The optimized artificial feeding chambers now allow us to collect ticks at precise intervals throughout their feeding stages, guided by their scutal index (Meiners et al., 2006). This advancement facilitates the study of tick biology during various feeding stages and ensures accurate and consistent data collection and experimentation. The molecular mechanisms and kinetics of tick cuticle expansion and the molting process have not been studied in detail. However, previous work has shown the morphological changes in the tick cuticle during animal feeding. Our analysis of the fully engorged nymphal alloscutal (abdominal) cuticle, obtained from ticks fed in artificial feeding chambers and animals demonstrate that they are similar at the ultrastructural level. This lays the groundwork for more in-depth mechanistic investigations into the tick cuticle. This optimized model will facilitate the collection of samples at specific time points, enabling an analysis of tick cuticle that includes assessing the expression of chitin synthase genes and the total chitin levels during cuticle expansion and the molting process. Furthermore, it will permit us to identify differences in male versus female cuticle development based on weight and scutal index determinations.

In summary, the newly enhanced tick feeding system will serve as an invaluable tool for advancing our understanding of tick cuticle biology.

CRediT authorship contribution statement

Faith Kozisek: Investigation, Methodology, Formal analysis, Funding acquisition, Writing – review & editing. **Jonathon Cenovic:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Savannah Armendariz:** Investigation, Methodology, Formal analysis, Funding acquisition, Writing – review & editing. **Subbaratnam Muthukrishnan:** Writing – review & editing, Conceptualization, Investigation. **Yoonseong Park:** Writing – review & editing, Conceptualization, Investigation. **Vinai C. Thomas:** Investigation, Writing – review & editing, Conceptualization. **Sujata S. Chaudhari:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

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