FROM NANO TO MICRO TO MACRO: IMPORTANCE OF STRUCTURE AND
ARCHITECTURE IN SPIDER SILK ADHESIVES

A Dissertation

Presented to

The Graduate Faculty of the University of Akron

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Vasav Sahni

August, 2012
FROM NANO TO MICRO TO MACRO: IMPORTANCE OF STRUCTURE AND ARCHITECTURE IN SPIDER SILK ADHESIVES

Vasav Sahni

Dissertation

<table>
<thead>
<tr>
<th>Approved:</th>
<th>Accepted:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advisor</td>
<td>Department Chair</td>
</tr>
<tr>
<td>Dr. Ali Dhinojwala</td>
<td>Dr. Ali Dhinojwala</td>
</tr>
<tr>
<td>Committee Member</td>
<td>Dean of the College</td>
</tr>
<tr>
<td>Dr. Gary Hamed</td>
<td>Dr. Stephen Z.D. Cheng</td>
</tr>
<tr>
<td>Committee Member</td>
<td>Dean of the Graduate School</td>
</tr>
<tr>
<td>Dr. Toshikazu Miyoshi</td>
<td>Dr. George R. Newkome</td>
</tr>
<tr>
<td>Committee Member</td>
<td>Date</td>
</tr>
<tr>
<td>Dr. Todd A. Blackledge</td>
<td></td>
</tr>
<tr>
<td>Committee Member</td>
<td></td>
</tr>
<tr>
<td>Dr. Jutta Luettmer-Strathmann</td>
<td></td>
</tr>
</tbody>
</table>
ABSTRACT

Spiders employ clever behavioral strategies combined with almost invisible custom-made adhesives for locomotion and prey-capture. The adhesive produced by modern orb-weaving spiders to capture prey (viscid glue) is laid on a pair of extensible axial silk fibers as micron-size glue droplets that are composed of a mixture of salts and polymeric glycoproteins. Each glue droplet is composed of a dense core surrounded by a sparse shell. We discuss the importance of the structure at nano-, micro-, and macro level in adhesion. At the nano level, we show that the inherent elasticity in the glue enhances adhesion caused by specific adhesive ligands by over two orders of magnitude. Furthermore, we describe how the viscoelastic solid nature of the glue drops help in capturing and retaining prey. We also develop an energy model to separate the axial silk contributions from glue droplet contribution in the force required to separate a whole thread from a surface.

We describe the functions of the salts that are present in large quantities in the web, and are nutritionally and physiologically essential for the spider. Previously, it was assumed that the main function of the salts is to sequester water. We show that salts play a major role in adhesion itself and how the core-shell microstructure developed within each drop facilitates reversible usage of this glue.
We compare the properties and humidity-responses of orb-weaving glue with the gumfoot glue produced by cob-weavers, the evolutionary descendants of orb-weavers and despite being produced in homologous glands, we find very interesting differences. We mimic the common macro-architecture of these capture threads: Beads-on-a-string (BOAS) architecture, and understand why spiders employ this architecture for capturing prey. Lastly, we discuss the attachment discs produced by spiders, which make it possible for spiders to move, defend, and capture prey. We comment on how the macro architecture of these attachment discs affects their adhesion, thus enabling different functions in a web.

This research, thus show the importance of structure at different length scales in influencing adhesion and shall inspire future efforts directed towards tunable adhesives.
ACKNOWLEDGMENTS

"Guru Govind Dono Khade
Kaake Lagoon Paaye
Balihari Guru Aapne,
Govind Diyo Bataaye” – Kabir Das

Roughly translated, this famous Hindi prose written by the great Kabir Das means that, if my Teacher and God himself were to stand in front of me, I would bow down to my Teacher first, because it were not for Him, I would have never known anything and I would not have known what God is.

That being said, I cannot put in words how much I am grateful to my advisor Prof. Ali Dhinojwala for everything that I have done during the entire course of my PhD. I thank him for giving me the excellent guidance, and the ability to think scientifically. He has been a great source of inspiration and a role model during the course of my graduate studies. He imparted the ability to tackle a given problem in the most simplistic manner. One of the important lessons I learned from him is to become the biggest critics of my own work before presenting it to the scientific community, which has really helped
me. When I met him for the first time and asked what my research would be, he said “do whatever you want”. He showed immense confidence and me and gave me a lot of freedom, which has helped me grow and made me an independent thinker. He has also given me wonderful opportunities outside of my dissertation research, and has helped me make contacts with professionals in different fields, which has given me a ‘multi-disciplinary’ perspective, something which was necessary for doing the kind of research that I have done. One of the most important things that I now share with Him, again thanks to Him, is the way I look at Science: Fun. This is the reason that despite working very long hours on a lot of days, I hardly ever feel stressed. I hope that I take this newly-acquired trait with me wherever I go. I have also learned from Him how to handle ‘conflicting situations’, which is really useful for a tactless ‘hot-head’ like me. He has also helped me a lot in my personal life for which I will be forever grateful.

I would also like to thank Dr. Todd A. Blackledge, without whom my research would not have been possible. Not only has he given me great guidance about the Biology and Evolution of spiders, he has also guided me on the materials and experimental aspects. One thing that I really admire about him is that he is very hands-on, always knows or has some ideas on how to do or get things done. It amazes me how he is always so energetic and, despite being tremendously busy with so much teaching, research, and, of course, family, and how prompt he is with his extremely helpful inputs and comments. He has also, knowingly or unknowingly, given me some very useful tips on how to make effective technical presentations, since he is great at them himself.
I also want to acknowledge Prof. Gary Hamed for his valuable suggestions and for taking the time to chair my Research Presentation and defense committee. I am extremely impressed by his huge knowledge base. He knows everything about everything and is always willing to discuss interesting spider silk problems and efficiently breaks down a complex problem into smaller easy-to-understand steps. He is a great teacher also.

I would also thank Dr. Jutta Luettmers-Strathmann. I have had very interesting discussions with her on and off about many different problems that I have faced. She is a great teacher too. Dr. Toshi Miyoshi has also been tremendously helpful with NMR. I thank him for taking all the time out to understand the spider silk problems and agreeing to work with us and giving us time on his, extremely busy, equipment.

I would also like to thank one man whom most of the graduating Polymer Science students thank: Mr. Ed Laughlin. He has set of magical hands, is very skillful with fabrications, and has helped me on a lot of occasions with small-device design and fabrication.

Over the years, I have worked with different high school, undergraduate, and masters students who have all been great and have taught me as much as they have learned during their time with us. I acknowledge Johanna Villate, Evelyn Ojo, Disha Labhasetwar, Chelsea Golias, Jared Harris, Vincenzo Volpe, Sinuo Long. I also thank all of my group members: Alyssa Stark, Adrian Defante, Mike Heiber, Sunny Sethi. Ila Badge, Yu-Tsan Tseng, Liehui Ge, Anish Kurian, Ping-Yuan Hsu, as well as the newer
I have learnt at least one trait from each one of my group members: courage from Anish, patience from Liehui, diligence from Alyssa, and so on. Also, I am really grateful to Cecilia Boutry for helping me with handling the venomous black widow spiders.

I bow my head in acknowledgement of almighty God who has blessed me at every step of my life. All of my hard work and dedication has been compensated with huge amounts of luck and opportunity.

Last, but certainly not the least, I would like to thank to the core of my heart my parents, who have supported me through everything I have wanted to do. Even when I left a highly lucrative and well-paying job to come and pursue my PhD in the US, my parents supported me. They have loved me and have taught me what love is. My life in the US would never have been the same if it were not for my brother. He is my sanity away from home. I would also like to thank all my friends in Akron and back home for all the good times that we have had.
# TABLE OF CONTENTS

**LIST OF FIGURES** ........................................................................................................... xii

**CHAPTER**

1. **INTRODUCTION TO ADHESION AND ADHESIVES**.................................1
   1.1 Timeline ............................................................................................................... 1
   1.2 Framework to understand adhesives and adhesion ........................................ 2
   1.3 Wetting of substrate/Bond formation ............................................................. 4
   1.4 Development of cohesion in the adhesive .................................................. 6
   1.5 Controlling adhesion ...................................................................................... 8
   1.6 Conclusion ........................................................................................................ 10

2. **NATURAL ADHESIVES** ..............................................................................11
   2.1 General Introduction .................................................................................... 11
   2.2 Biofilms ......................................................................................................... 13
   2.3 Marine mussels ............................................................................................ 14
   2.4 Gastropod ....................................................................................................... 15
   2.5 Australian Frogs ............................................................................................ 16
   2.6 Geckos ........................................................................................................... 16

3. **SPIDER SILK ADHESION** ........................................................................ 18
   3.1 Abstract .......................................................................................................... 18
   3.2 Introduction ...................................................................................................... 18
3.3 Spider Webs ......................................................................................................19
3.4 Bolas Spider ......................................................................................................23
3.5 Brown Recluse Spiders ....................................................................................25
3.6 Black Widow Cobwebs ....................................................................................25
3.7 Orb-weaving spiders ........................................................................................29
3.8 Adhesion of cribellar and viscid threads ........................................................41

4. VISCOELASTIC SOLIDS EXPLAIN SPIDER WEB STICKINESS....................50
   4.1 Abstract ............................................................................................................50
   4.2 Introduction .......................................................................................................50
   4.3 Methods ............................................................................................................51
   4.4 Results .............................................................................................................53
   4.5 Discussion ........................................................................................................57

5. SPIDERS USE ‘SALTY SILK’ TO CAPTURE PREY ...........................................62
   5.1 Abstract ............................................................................................................62
   5.2 Introduction .......................................................................................................63
   5.3 Methods ............................................................................................................64
   5.4 Results .............................................................................................................66
   5.5 Discussion ........................................................................................................74

6. CHANGES IN THE ADHESIVE PROPERTIES OF SPIDER AGGREGATE GLUE DURING THE EVOLUTION OF COBWEBS ..................................................79
   6.1 Abstract ............................................................................................................79
   6.2 Introduction .......................................................................................................80
   6.3 Methods ............................................................................................................82
   6.4 Results .............................................................................................................85
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Different kinds of prey capturing strategies. Optimization of spider web architectures on an evolutionary tree of spiders. Adapted from [49]</td>
</tr>
<tr>
<td>3.2</td>
<td>Bolas Spider. A bolas spider with its web – a gluey blob at the end of a single silk line that it swings to catch its moth prey. Adapted from <a href="http://nathistoc.bio.uci.edu/spiders/Mastophora.htm">http://nathistoc.bio.uci.edu/spiders/Mastophora.htm</a></td>
</tr>
<tr>
<td>3.3</td>
<td>Ribbon silk. Figure shows an SEM image of the major ampullate silk spun by female L. laeta. The silk is unique in its highly anisotropic shape, compared to the relatively cylindrical silk of most other spiders. Arrows point towards the plaque of adhesion between ribbons. Scale bar is 10µm. It is adapted from [56]</td>
</tr>
<tr>
<td>3.4</td>
<td>Cob webs. A schematic of a cob-web. Adapted from [59]</td>
</tr>
<tr>
<td>3.5</td>
<td>Cob webs. Cob webs can capturing flying prey as well as walking insects. When a walking insect contacts a sticky gum-footed thread, it quickly detaches from the substrate and yanks the insect into the air. Figure shows a simulation of this using a white mass instead of an actual insect. It is adapted from [60]</td>
</tr>
<tr>
<td>3.6</td>
<td>Orb-web. Figure shows a typical orb-web.</td>
</tr>
<tr>
<td>3.7</td>
<td>Cribellar Silk. a) A completed cribellar thread often forms a series of regularly spaced puffs that are brushed into place by the spider’s legs. The surface of these threads is made up of thousands of nanofibers. These nanofibers can be either non-noded (b) or noded (c). It is adapted from [65]</td>
</tr>
<tr>
<td>3.8</td>
<td>Viscid silk. SEM (JEOL) image of a completed viscid capture silk spun by <em>Lariniodes cornutus</em>.</td>
</tr>
<tr>
<td>3.9</td>
<td>Viscid silk. This kind of a capture silk is spun using a triad of spigots. Axial silk is spun in the flagelliform (FL) gland after which it is coated with aqueous glue produced in the aggregate (AG) glands as shown in (b). Figure is adapted from [49]</td>
</tr>
<tr>
<td>3.10</td>
<td>Structure of the glue droplet. a) Figure shows a schematic of the hypothesized three-phase model of the glue droplets. It is adapted from [84]</td>
</tr>
<tr>
<td>3.11</td>
<td>Compound light microscope photographs of A. marmoreus (A) and A. aurantia (B). Scanning electron micrographs of combined A. marmoreus and H. cavatus threads (C), a H. cavatus thread (D), and combined A. aurantia and H. cavatus threads (E), showing an</td>
</tr>
</tbody>
</table>
unicorporated cribellar thread region (UCR) and a composite thread region (CTR). Adapted from [92].

3.12 | Extensibility plays a huge role in adhesion. Figure shows a schematic of the difference in the thread adhesion behavior when a cribellar thread and a viscid thread are detached from a surface. The difference in the axial silk extensibility in both the cases can be attributed partly to this effect. It is adapted from [90].

4.1 | Single drop adhesion experiments. Figure a shows the components of the capture thread of the spider Lariniodes cornutus: 1. Viscous coat, 2. Glycoprotein granule, and 3. Axial thread. Scale bar = 20 µm. Recently, it has been suggested that the viscous coat contains transparent layers of glycoproteins [84]. Inset shows a schematic of the single drop pulling experiments wherein single glue drops (green) of a capture thread were stretched using a conical glass probe (blue) while the force responses were recorded. Figure b shows the conical glass probe approaching a single glue drop. Scale bar = 50 µm. Figure c shows stretching of a single glue drop using the conical glass probe. Scale bar = 60 µm. Figure d shows the force responses when single glue drops were stretched at different rates until separation from the glass probe. The curves are plotted as mean ± standard deviation from three measurements each (error bars are in black for all the three cases).

4.2 | The adhesive forces are dominated by elasticity rather than capillary forces. Figure a compares the pull-off forces at different stretching rates for glue drops (red markers) with the capillary forces exerted by PDMS melt (Mw. = 6000 Da, Me = 8000 Da, γ = 19.8 mN/m, green markers) and the calculated capillary forces exerted by the viscous coat (γ = 40 mN/m). For PDMS-glass and viscous coat-glass, the contact angle Θ was taken as 0. The values are plotted as mean ± standard deviation from three measurements each. Figure b shows load-relaxation curves in which the glue drops were stretched by 100 µm at three different rates (100µm/s (black), 10µm/s (blue), 1µm/s (red)) after which the load was allowed to relax. Inset shows an enlarged view of the plateau regions for the three cases. All the measurements were performed close to 25°C at 40% R.H. The curves are plotted as mean ± standard deviation from three measurements each.

4.3 | a and b show transmission mode images of glycoprotein granules at 90% R.H. and 0% R.H., respectively. Scale bar = 20µm.

4.4 | Peeling capture silk thread from the surface depends on the peeling rate. (a) The effect of the rate of pull-off (dh/dt) on the force at pull-off (blue markers and black error bars). Values are plotted as mean ±s.d. from 15 measurements each. (b) Adhesion energies at two pull-off rates using the model explained in the text. (c) The stress – strain behaviour of capture spiral thread at the rate of 2 mm s⁻¹ (red) and 0.1mm/s (blue). The curves are plotted as mean ± s.d. from five measurements each. Error bars are in black for both cases. Measurements were taken at 40 % RH close to 25 °C. (d) Repeatability of the force at pull-off of a capture thread of L. cornutus. Measurements were taken close to 25 °C at 40 % RH. The pull-off rate was 2 mm/s.
4.5] Energy model. Figure shows the total work (black) done in separating the thread from the surface, strain energy stored in the axial silk (blue), and the energy contribution of the glue drops (green) when the thread went through the consecutive stages shown in inset 2, 3, and 4. The geometry used for the pull-off measurements is shown in the inset Figure 1. Calculation of $U_{\text{strain}}$ and $U_{\text{glue}}$ involved the reasonable approximation that $D << L$. In these experiments this condition is satisfied ($0 \text{ mm} \leq D \leq 2 \text{ mm}$ and $L = 16 \text{ mm}$). The final values of $U_{\text{strain}}$ and $U_{\text{glue}}$ do not depend on the condition that $D << L$.

5.1] Effect of Salts on the force of adhesion of capture threads. Figures A and B show the adhesion values for pristine and washed capture threads under different conditions. Notice the difference in the units for both the figures. P and W indicate pristine and washed capture threads, respectively. Inset in Figure A shows the set up used to measure the force of adhesion of single capture threads with a 2 mm wide clean glass plate. The adhesion forces for the washed threads (W) are usually two orders of magnitude less than the pristine threads (P). The difference in the stickiness units between figures A and B has been emphasized using dotted lines. Since the results shown here are pair-wise comparisons, the difference in adhesion values cannot be due to difference in the salt composition of the glue droplets.

5.2] Effect of salts on the energy of adhesion of the glue drops. Figure 5.2A shows the energy of adhesion of the glue drops of W-threads under different conditions, calculated using the energy model developed earlier by us. Energy of adhesion of glue drops of W-threads is independent of humidity even though the forces of adhesion increase with humidity (Figure 5.1A), implying that the difference in forces of W-threads at different humidity is due to the axial silk fibers becoming softer and more extensible with humidity. Figure 5.2B shows the energy of adhesion of P- and W-threads under different conditions. The huge difference between the energies of P- and W-threads for the same conditions indicates that the difference in the force of adhesion for these threads is due to the difference in the adhesion characteristics of the glue drops and not just due to the difference in the tensile characteristics of the P-and W-axial threads. Interestingly, P- and W-threads share very similar tensile mechanics which vary in the same fashion with humidity and rate of pulling. The difference in the energy units between figures A and B has been emphasized using the dotted lines.

5.3] Effect of salts on the humidity-response of the glue drops. Figures A and B show optical images of the single glue drops of P_0 and P_100, respectively, and figures C and D show single glue drops of washed capture threads in air (equilibrated at 0% R.H.) and under water, i.e. W_0 and W_wet, respectively. Pristine glue drops swell when exposed to humidity while washed glue drops remain unaffected even when submerged in water. Scale bar for every image is 50 µm. All measurements were performed at ~ 25ºC.

5.4] Re-introduction of Salts. Figures A and B show a washed silk thread and the washed silk thread immersed in a salt solution consisting of choline, glycine betaine, and N-acetyltaurine, and isethionic acid respectively. No changes in the size and shape of the washed silk were observed upon removing the thread from the salt solution after three hours of adding the salt back (Figure C). Figure D shows a droplet suspended from a
pipette tip collecting salts from a pristine silk thread. 1, 2, and 3 in Figure D show a pristine silk thread, water droplet, and the pipette tip. Upon immersing a washed thread in the same water drop (Figure E), no changes in size and shape of the glue droplets were observed (Figure F). Figure G shows a washed silk thread which was overlaid onto a pristine silk thread to form a composite thread (Figure H). 1, 2, and 3 in Figure H show a washed glue drop, a pristine silk thread, and the glue of the pristine silk thread, respectively. Upon separating the washed thread from the composite thread, no changes were observed in the washed glue droplets (Figure I). Scale bar = 25 µm.......................

6.1| Gumfoot silk glue vs. viscid silk glue (a) and (b) show individual viscid silk thread and gumfoot silk thread spun by Lariniodes cornutus and Latrodectus hesperus, respectively. Capture threads were laid on clean cover slips for both the cases. The difference in the wetting kinetics of the coating peptides and the high-molecular-weight adhesive polymers (probably glycoproteins) gives the appearance of a ‘diffuse core’ in the gumfoot silk glue droplets. The glue droplets homogenize with time which disappears the core. Also, this core is not observed in pictures of suspended gumfoot silk threads. Scale bar is 20 µm for both the cases. (c) and (d) show a gumfoot silk thread at 0% R.H. and 90% R.H., respectively. It was observed that when a gumfoot silk thread is humidified, the glue droplets flow and coalesce to form bigger droplets..................... 87

6.2| Water uptake of the glues. Change in volume of the viscid silk glue (squares) and gumfoot silk glue (circles) as the silk threads are exposed to a high-humidity environment. Insets a (c) and b (d) show gumfoot silk glue (viscid silk glue) at 0% R.H. and 100% R.H., respectively. Similar to figures 6.1c and 6.1d, inset b shows fewer but bigger glue drops than inset a. Scale bar is 100 µm for all the figures. The uptake of water in viscid silk glue drops is due to the presence of low molecular weight hygroscopic compounds present in the glue. It was experimentally determined that there is no hysteresis in water uptake with humidity cycling (data not shown). In the case of the gumfoot silk glue, however, the order of changing humidity plays a role. While going up in humidity for the first time, the glue drops on gumfoot silk coalesce to form bigger drops and a slight change in total glue volume is observed (circles). Reducing the humidity subsequently restores the original glue volume but obviously not the original number of glue drops. Subsequent humidity cycles are completely reversible in terms of both glue volume and number of glue drops. ................................................................. 88

6.3| Effect of humidity on the stretching behavior of the glues. Force-displacement behavior when glue drops of viscid silk (gumfoot silk), equilibrated at 15% R.H. a(b), 40% R.H. c (d), and 90% R.H. e (f), were stretched at 1 µm/s (inverted triangles), 10 µm/s(upright triangles), 50 µm/s(squares), and 100 µm/s (circles)...............................

6.4| Comparison between viscid silk glue and gumfoot silk glue. (a) and (b) Force-displacement behavior when individual glue drops of viscid silk and gumfoot silk, equilibrated at 15% R.H. (circles), 40% R.H. (squares), and 90% R.H. (upright triangles), are stretched at 50 µm/s, respectively (data acquired from Figure 2). (c) Comparison of the pull-off force obtained from Figure 4a and b with the capillary forces exerted by unentangled PDMS (γ ~ 20 mN/m) and an aqueous solution of composition similar to the
viscous coat used by modern orb-weaving spiders to coat their capture threads ($\gamma \sim 40$ mN/m). VSS glue denotes viscid spiral silk glue whereas GFS glue denotes gumfoot silk glue. VSS glue, depending on the relative humidity, is represented by box and whiskers outlined by red (15% R.H.), blue (40% R.H.), and green (90% R.H.), whereas, for GFS glue, boxes and whiskers are outlined with black and boxes are filled with the color. PDMS is represented by box filled with purple whereas aqueous solution is represented by box and whiskers outlined with purple. d) Comparison of energy of adhesion between viscid silk glue, gumfoot silk glue, and the $U_{\text{glue}}$ values obtained using the energy model as explained in the supplementary information. Values are obtained by multiplying the area under the force-displacement curve obtained from individual glue drop stretching measurements by 42 (number of glue drops in contact with a 2 mm glass substrate used for the peeling experiments). Even though gumfoot silk does not have 42 droplets per 2 mm length, and thread peeling measurements were not performed with it, values plotted are obtained by multiplying the area under the force-displacement curve by 42, in order to compare it with viscid silk glue and the $U_{\text{glue}}$ values obtained using the energy model. Values are plotted as box and whiskers from 5 measurements each. VSS glue is represented by box and whiskers outlined with black and filled with red (1 µm/s), blue (10 µm/s), green (50 µm/s), and purple (100 µm/s). GFS glue, depending on the rate of stretching, is outlined by one of the above colors. $U_{\text{glue}}$ values are represented by brown-filled boxes outlined with black.

6.5| Peeling of capture silk threads is humidity-dependent. (a) Schematic of the set-up used for thread peeling measurements. (b) Peeling forces when capture threads, equilibrated at different values of R.H., were separated at 2 mm/s from a 2 mm wide clean glass substrate. Values are plotted as box and whiskers from 10 measurements each. (c) Tensile characteristics of capture threads equilibrated at 0% R.H. (circles), 15% R.H. (squares), 40% R.H. (upright triangles), 65% R.H. (inverted triangles), and 90% R.H. (diamond). Stretching rates in each case were similar to the stretching rates of capture threads during peeling measurements. Values are plotted as mean ± s.d. from 5 measurements each. (d) Energy consumed by all the glue droplets (in contact with a 2 mm glass substrate) in stretching during thread peeling measurements at different values of R.H. Values are determined using the energy model as has been described in the text and are plotted as box and whiskers.

6.6| Effect of humidity on crosslinkers. Load-relaxation behavior of individual glue drops of viscid silk (gumfoot silk) equilibrated at 15% R.H. a (b), 40% R.H. c (d), and 90% R.H. e (f) stretched by a constant length at rates of 1 µm/s (inverted triangles), 10 µm/s (upright triangles), 50 µm/s (squares) and 100 µm/s (circles). Values are plotted as mean ± s.d. from 5 measurements each. When viscid silk glue is stretched at 100 µm/s at 15% R.H., it releases contact with the tip before stretching 100 µm (Figure 3a), hence load relaxation measurements could not be performed at these conditions. (Figure 6.6a)....

6.7| Effect on glue elasticity. Plateau values, indicative of the amount of elasticity in the glue, reduce with increasing humidity in the case of viscid silk glue (a) but remain constant for gumfoot silk glue (b). Plateau values for gumfoot silk glue are plotted using
a fitting function since the values were lower than the resolution of the equipment (1µN).

6.8| Polymer model to understand the humidity effect. (a) Pull-off energy plotted as a function of concentration of the PEO/water solution at a pull-off rate of 1mm/sec. (b) Energy calculated as area under the load-displacement curve during pull-off plotted as a function of the pull-off rate for concentrations of 13.7% (circle), 17.7% (upright triangles), 35.5% (squares), and 52.2% (inverted triangles) of the PEO/Water solutions. (c) A schematic of the state of the glue drops at different values of R.H. Chemical crosslinking (red) remains unaffected with changes in humidity while the viscosity and elasticity reduce with increasing humidity. Lubricating action becomes predominant at higher values of humidity.

7.1| Fabrication and tuning of ‘functional-threads’. a) and b) show a capture spiral thread spun by Argiope trifasciata and a thread produced by withdrawing a nylon thread out of a reservoir filled with PDMS, respectively. c) Shows the effect of velocity of coating on drop dimensions when nylon threads are coated with PDMS of kinematic viscosity 1000 cst at velocities of 690 µm-s\(^{-1}\), 2460 µm-s\(^{-1}\), and 9460 µm-s\(^{-1}\) (left to right). d) Shows the effect of PDMS viscosity on drop dimensions. Nylon threads are coated, at 9460 µm-s\(^{-1}\), with PDMS of kinematic viscosities 10 cst, 100 cst, and 1000 cst (left to right). Capillary number increases from left to right. Scale bars in c and d are 150µm and 50µm, respectively.

7.2| Testing of functional threads. a) Shows the suspension bridge formed when a capture silk thread spun by Larinioides cornutus is separated from a glass surface. Interestingly, the functional-threads produced by coating Nylon threads with PDMS behave similarly and also exhibit the formation of a suspension bridge-like structure when separated from a substrate (shown in Figure 7.2b). c) Shows the adhesion force of a functional thread (PDMS-coated nylon thread) as a function of the capillary number (Rate of separation of thread from the substrate = 2 mm-s\(^{-1}\)). d) Shows the effect of capillary number on the adhesion energy of the PDMS drops. The energies were determined using a recently developed energy model5. The error bars are calculated from the error in \(W_T\) and the \(U_{\text{Strain}}\) from 30 measurements each.

7.3| Schematic of the Set-up. Figure shows a schematic of the set-up used for adhesion. The dotted line bridging the gap between the cardboard legs shows the initial position and unstrained length \(l = 16\) mm of the capture/functional thread. The width of the clean glass plate fixed onto the lower clamp is 2 mm. The thread is pushed onto the glass plate to a force of 20 µN at a rate of 0.1 mm s\(^{-1}\) (displacement-controlled loading), held there for 60 seconds, and then pulled away from the substrate at a fixed rate (2mm/sec for Figures 2c and d, and 0.5mm/sec and 2mm/sec for Figure 3 inset), while the force-displacement response (\(f(h)\)) is recorded every 0.001 seconds. Force is measured by the displacement of the actuating transducer. Just before the thread releases contact with the substrate, the last few drops (green) in contact with the glass plate stretch and give the appearance of a suspension bridge, as shown in the schematic (stretching of glue drops is exaggerated, compared to \(h_0\), for the sake of visibility). The force registered just before
these last drops release contact with the class plate \( f(h) \) is recorded as the pull-off force (adhesion force shown in figure 7.2c). Figures 7.2a and 7.2b also show suspension bridges formed by spider capture silk and functional threads, respectively. ........................ 114

7.4 | BOAS versus Cylindrical morphology. Shows the contact area established on glass by equal volumes of cylinder and the eventually formed droplets, calculated assuming JKR theory which is applicable here since the nylon fiber is coated with PDMS (Sylgard 528 A and B) which, after crosslinking, becomes elastic. The volumes of the cylinders and spheres were calculated using volume conservation on the dimensions of the glue drops on the capture spiral threads spun by Cyclosa turbinata (●), Leucauge venusta (■), Metepeira labynthia (▲), Araneus pegnia (▼), Argiope trifasciata (◄), and Araneus Marmoreus (►)12. Solid symbols represent spheres whereas the corresponding hollow symbols represent cylinders. Spheres establish higher contact area than cylinders for the same loading force. Inset in Figure 7.4 compares the adhesion of freshly spun capture threads (the coating is still cylindrical) (hashed bars) versus capture threads in which the coating has beads-on-string structure (solid bars), at different rates of pull-off (speed at which the thread is separated from the substrate). Glue droplets cause the capture threads to adhere many times stronger than the cylindrical glue coating........................................ 118

8.1 | Attachment disc morphology matches different functions. Figure shows SEM images of scaffolding disc (a) and gumfoot disc (b) spun by the cobweb-weaving spider Achaearanea tepidariorum.Insets show optical microscope images of the respective discs. The black arrows point at dragline silk (MA silk) in Figure a and its inset, while the white arrows point at pyriform fibers, arranged in a ‘staple-pin’ architecture, attaching the dragline silk to the surface. Black arrows point at gumfoot thread (MA silk covered with aggregate glue) in Figure b and its inset while the white arrows point at pyriform fibers, arranged in a dendritic architecture, attaching the gumfoot thread to the surface. Scale bars in both the figures are 100 µm. ........................................................................ 127

8.2 | Pyriform fibers have two components. Figures 8.2a and 8.2b show the extremities of a scaffolding disc and a gumfoot disc spun by Achaearanea tepidariorum. Pyriform fibers are a composite system containing an axial fiber coated with a fluid. The black arrows point towards the fluid. The white arrows in Figure S1a point towards the hair-pin bends at the extremity of a scaffolding disc indicating that the spider’s spinnerets sweep back and forth, across an MA silk fiber, with their pyriform glands when spinning these discs. Scale bar in both these images is 5 µm........................................ 128

8.3 | Pyriform fibers suspended in air in a gumfoot disc. Figure shows an SEM image of a gumfoot disc spun by Achaearanea tepidariorum. The white arrows point at the pyriform fibers that are suspended in the air thereby reducing contact with the surface. The black arrows point towards the fibers that are attached to the surface. A gumfoot disc is attached to the surface only at its periphery. Moving towards the periphery, pyriform fibers split successively, such that fibers that are attached to the surface have the finest diameters. The scale bar in this image is 20 µm........................................ 130
8.4| Sample preparation for adhesion measurements. Figure a shows the cobweb-weaving spider *Achaearanea tepidariorum* spinning a scaffolding disc on a nylon thread. The inset shows an optical microscope image of a scaffolding disc spun on a nylon thread (30 μm diameter). The black arrows point towards the nylon thread in both Figure a and its inset. Figure b shows a schematic of a typical cobweb. The black arrow points towards the flag used to collect individual gumfoot threads with the attached pyriform attachment disc (light green).

8.5| Adhesion measurements. Figure a shows the force required to separate a gumfoot disc and a scaffolding disc from a clean glass surface. Gumfoot disc adhesion is an order weaker than scaffolding disc adhesion. The force required to break a dragline silk (MA silk) thread is also plotted. A dragline silk thread breaks when it is pulled to peel a scaffolding disc (hence thicker nylon threads were used to peel scaffolding discs). Gumfoot discs were peeled using the gumfoot silk threads (four intertwined MA threads). In theory, however, gumfoot discs could be peeled using just one MA thread since the breaking force of MA thread is almost twice the adhesion of gumfoot discs with glass. This indicates the scaffolding disc adhesion is much stronger than gumfoot disc adhesion. Figure b shows the energy of adhesion of a gumfoot disc and a scaffolding disc. Gumfoot discs adhere an order weaker than scaffolding discs. The energy of adhesion is determined by subtracting the strain energy contribution of nylon threads (while peeling scaffolding discs), and gumfoot threads (while peeling gumfoot discs) from the total work done in peeling the discs.

8.6| Modelling the discs. Figure a shows a schematic of the simulated scaffolding disc. The length $l_{sp}$ is 100 mm while the width $w_{sp}$ is such that $6*w_{sp} = 36$ mm. The inset shows how the peeling measurements were conducted. The nylon strand is pulled on perpendicular to the plane of the tapes while the load-extension behaviour is registered. Figure b shows a schematic of the simulated gumfoot disc. The length $l_{rp}$ and the width $w_{rp}$ of the tape strips is equal to $l_{sp}$ and $w_{sp}$, respectively. All the six strips are firmly secured to the strand of nylon threads at the center of the circle. The insets on the left and right show the top and front views of the modelled tapes. Figure c shows the load-extension results obtained from the adhesion measurements of the simulated scaffolding discs. Circles denote the case with length = $l_{sp}$ and width $w_{sp}$, squares: $l_{sp}/2$, $w_{sp}$, and triangles: $6*w_{sp}$, $l_{sp}$. The inset shows a plot comparing the adhesion forces of simulated scaffolding silk with the simulated gumfoot disc, both using the same dimensions of the strip: $l_{sp}$, $w_{sp}$. Figure d shows the load-extension results obtained from the adhesion measurements of the simulated gumfoot discs. Circles denote the case with length $l_{rp}$ and width $w_{rp}$. Squares denote length ~ $l_{rp}/2$ and width $w_{rp}$, upright triangles: length $l_{rp}*3/4$ and width $w_{rp}/2$, and inverted triangles: width $w_{rp}$ and length ~ 3mm.

8.7| Dimension-dependence of peeling forces. Figure S3a shows the load-extension response obtained from peeling of simulated scaffolding disc. The length of the strips is $l_{sp}$, width $w_{sp}$, but the thickness is twice from Figure 8.6c. Change in thickness affects the peeling force. Figure b shows the load-displacement response obtained from peeling of simulated gumfoot discs. For filled green squares, the length of the strips is $l_{rp}$, width $w_{rp}$, but the thickness is twice from Figure 8.6d. Thickness has no effect on peeling force.
here. For filled green diamonds, length is $l_{rp}$, thickness is same as in Figure 4d, but the width goes from $w_{rp}$ at the proximal end to 1 mm at the distal end, with a linear gradient. This gradient best represents the gumfoot discs.

8.8 Evolutionary convergence in using architecture to mediate attachment disk function. Orb-weaving spiders are the evolutionary ascendants of cobweb-weaving spiders. Figure shows a staple-pin attachment disc spun by the orb-weaver *Larinioides cornutus*, architecturally similarly to the scaffolding disc spun by cobweb-weavers (Figure 1a). This disc is used by orb-weavers to attach their webs and draglines to different surfaces, thus facilitating prey-capture and locomotion, respectively. The chemical composition of this disc has been shown to be different from that of scaffolding disc of cob-weavers, but the architecture is identical. This disc, like the scaffolding disc of cob-weavers, binds stronger than the breaking force of an MA fiber, which has important consequences in web-engineering, as explained in the text. The scale bar is 100 µm.
INTRODUCTION TO ADHESION AND ADHESIVES

Adhesion is a multi-billion dollar industry based on a truly multi-disciplinary science. Physicists, chemists, and mathematicians have, with their outstanding contributions for hundreds of years, developed this science. Lately, emphasis has also been laid upon the importance of specialized sciences like rheology (including contact and fracture mechanics), polymer chemistry, and surface chemistry, in this field of adhesion. This chapter will describe in short a timeline of adhesives and adhesion followed by a brief framework designed to understand different kinds of adhesives.

1.1 Timeline

The basis of a science that was developed by physicists, chemists, and mathematicians was discovered by Archaeologists when they discovered that tree sap was the first material ever to be used as an adhesive 6000 years ago [1]. This glue has also been found in various roles in temples, paintings, caskets. Some of the examples of this pre-historic glue can still be seen in Museums today. The next class of adhesives used was derived
from animal sources, 2000 years ago. Procedures describing production and usage of the 
glue were written. These glues were heavily used in bonding of thin sections or layers of 
wood [2].

Development of animal and fish glues was, in a sense, a landmark in the field of 
adhesives, since it led to the development of adhesives based on natural ingredients such 
as blood, bones, milk, cheese, vegetables and grains [3]. This was the first time that 
different materials were used to tailor adhesives to hold different surfaces together. A 
technological nightmare: adhesives that are water-resistant, were developed by the 
Romans when they used beeswax to caulk their boats [4]. From then till about 300 years 
ago, glues had attained widespread importance and were used in producing everything 
from furniture to weaponry to musical instruments [1].

The initiation of glue as an industry took place in the 1700s when animal glue was first 
produced in a commercial factory in Holland. Consequently, there have been some rather 
rapid developments in the field of adhesives. The first fish glue patent was issued shortly 
thereafter, followed by patents on other natural materials like casein, starch, and even 
natural rubber. The industrial revolution witnessed the United States producing several 
glues commercially. The first ever plastic polymer was synthesized around the same time 
[5]. This triggered the production of many adhesives based on synthetic plastics and 
rubbers.

A broad understanding of the working of adhesive together with the flexibility and 
freedom provided by the ability to synthesize adhesives helped the adhesion scientists
tremendously, since now different properties like setting time, temperature, toughness, chemical activity could be tailored to suit different needs [6].

Today adhesives are used in the production of almost all commercially available products, in one way or the other. Their applications are also increasing by the day. In addition to the afore-mentioned examples, adhesives are also used biologically: wound healing, dentistry, orthopedic surgery etc. A disadvantage experienced by most synthetic adhesives is that they are weakened by water. One of the ways to solve this problem is to resort to nature; barnacles and mussels and other under-water organisms can stick to anything. In a way, the science of adhesion has completed one whole circle; it started with nature and biomaterials and now, after 6000 years, is again going back to it. [1, 7].

1.2 Framework to understand adhesives and adhesion

Since an adhesive is generally defined as something which can join surfaces and resist separation with a significant force, the basic framework developed here deals with these two essential phenomena. First, the wetting of substrate: an adhesive must ideally establish atomic and molecular level contact with the substrate of choice. Secondly, the adhesive must resist separation: ideally speaking, for a strong joint, an adhesive must dissipate maximum energy at the time of separation [8]. To achieve these two processes, in most cases, the adhesive is applied at a low viscosity so that it spreads well, and then hardens into a cohesive unit so that it resists separation.
These two requirements clearly insinuate fact that adhesion is not just a surface phenomenon. The bulk of the adhesive plays an equally important, if not more, role in imparting high adhesion. This chapter covers these two phenomena in slight detail.

1.3 Wetting of substrate/Bond formation

Since most adhesives, except pressure-sensitive adhesives, are applied at low viscosity, they quickly spread onto a surface, establish intimate contact, and “attach” (form different kinds of bonds) with the substrate [9]. The nature of this process is such that there is a kinetic and a thermodynamic aspect to it.

To facilitate spreading, adhesives are either applied as a solution in a volatile solvent (so that it can be evaporated later to harden the adhesive), or as a melt that can later be cooled down (melt viscosity is low), or certain additives are added to reduce viscosity and improve flexibility. Since the adhesive is required to flow at this stage, it is not crosslinked yet, since crosslinked materials do not flow [10].

Once an intimate contact area is established, the adhesive bonds with the surface. There are different ways in which bonds, weak or strong, are formed between the adhesive and the surface. The most fundamental amongst these is physical adsorption, because it depends on van der Waal’s forces [11]. Van der Waal’s forces originate from the interactions between permanent dipoles (Keesom force), permanent and induced dipoles (Debye force), and between transient dipoles (London dispersion force). Since it is dependent on van der Waals forces, its effects are observed in all adhesives. A lot of
natural systems benefit from this interaction, even though the interaction energy is weak. Gecko’s remarkable ability to climb vertical walls is due to this phenomenon [11, 12].

Unlike physical adsorption, which is applicable to almost all adhesive systems, inter-diffusion is limited to only certain systems. As the name suggests, this theory postulates that, in certain adhesive systems of high molecular weight, the polymer chains diffuse with each other. This results in the formation of a thick inter-phase as opposed to a system that just relies on physical adsorption which forms a clear thin boundary. For the diffusion to happen, polymer systems must be mobile, so their application temperatures must be significantly higher than their glass transition temperatures. This phenomenon is widely observed in systems in which the adhesive is applied to both surfaces to be joined, and the surfaces are hot-pressed together. Here, the adhesives on the two surfaces can be same (which causes ‘autohesion’) or can be different. Care should be taken in making sure that the two adhesive systems are thermodynamically compatible. This is judged by considering their solubility parameters [13].

Another widely observed theory is the chemical bonding theory which postulates that, in certain adhesives systems, the formation of chemical bonds takes place across the adhesive-substrate interface. These bonds include covalent bonds, ionic bonds, and hydrogen bonds. Hydrogen bonding is widely observed in systems rich in –OH groups. Natural adhesives involve a significant amount of hydrogen bonding. The propensity of a system (adhesive and a substrate) to make covalent or ionic bonds is judged from the parameters $E_A$, $E_B$, and $C_A$, $C_B$, which describe the susceptibility of an acid (A) and its corresponding base (B) to undergo electrostatic (E) or covalent (C) interactions. Knowledge of this phenomenon enables polymer chemists to synthesize polymers with
specific functional group as branches to facilitate adhesion with a particular substrate [14].

A theory which is seldom observed in most polymeric adhesives is the electrostatic theory which postulates that when two surfaces are in contact, electrons are transferred from one to another resulting in the formation of an electron double layer that enhances adhesion [8]. This theory can explain metal adhesion since metals are conductors but fails to explain polymer adhesion since most polymers are insulators.

All the above theories are for the ideal case: smooth surfaces. In reality, almost no surface is completely smooth such that the true area of contact between an adhesive and a surface is much higher than the projected area. This leads to major enhancement of adhesion forces. Knowledge of this has led to the development of surface roughness with re-entrant angles such that when an adhesive flows into it and hardens or sets, it is rarely separated at the interface because of the inherent design of the asperities and mostly a cohesive failure is observed, leading to appreciation of adhesive forces [15].

1.4 Development of cohesion in the adhesive

Upon proper application of the polymeric adhesive, it undergoes a certain physical or chemical reaction that results in enhanced cohesion (referred to as ‘hardening’ in this chapter) in the adhesive bulk. Depending on the application, the time required for hardening can be short or long. A medical adhesive, for example, cannot harden immediately because the surgeon may need to adjust its position. On the other hand, it cannot take very long to harden too, since that would increase surgery time, chances of
infection, and, of course, the cost of the surgery. The hardening time, thus, is a key aspect of adhesion. Another aspect that is taken care of is the release behavior of the adhesive. When designing an adhesive system, a brittle failure or a ductile failure, can be almost predicted with the interplay between interfacial bonds and cohesive strength. These aspects are kept in mind while deciding how the particular adhesive will harden and how much it will harden.

An adhesive can harden by solvent evaporation. Usually, the solvent chosen is volatile so that hardening is time efficient and heat energy does not have to be supplied. The viscosity of the adhesive can be easily controlled at the time of application. However, hardening by solvent evaporation is associated with change in volume that has to be dealt with. Another hardening method is cooling. This is applicable to hot melt adhesives. Molten adhesive ‘flows’ and can thus establish good contact with the substrate, after which it is cooled down which causes it to harden. This process, due to its innate nature, is not energy efficient [16].

Crosslinking the adhesive after application is one of the most widely used hardening method. Crosslinked systems do not flow, so creeping of adhesives is not an issue. Also, depending on the adhesive system, crosslinking can take place at room temperature or can be precipitated by heating the system. So, the hardening time can be easily controlled. Another parameter in control here is the density of crosslinking. A very densely crosslinked system, like that seen in some structural adhesives, or a relatively lightly crosslinked system, like seen in pressure sensitive adhesives, can be achieved [17]. Covalent crosslinking can be induced by certain additives or by functionalizing the polymer itself. However, a lot of natural adhesives have very dense physical crosslinking
too. Ionic or hydrogen bonds act as physical crosslinks. These bonds are weakened with water and its vapor such that if the adhesive is cleverly designed, a combination of covalent and physical crosslinking can impart the adhesive the desired behavior.

Finally, certain polymeric systems, like the acrylates, are hardened by polymerization. These systems are applied in their monomeric or oligomeric forms so that the low viscosity facilitates wetting of the substrate. The polymer is then polymerized. Usually, the trigger for polymerization is simple and relatively abundant, like water and its vapor, in the case of cyanoacrylates. The rate of polymerization, and thus the hardening time can be controlled by varying the abundance of the trigger. If flow is to be kept to a minimum, tri-functional monomers are used, so that upon polymerization, a crosslinked network is formed [18].

1.5 Controlling adhesion

Fracture strength of an adhesive system, \( G \), depends on the interfacial bond strength, \( G_0 \), and an energy dissipation \( f \), which depends on the rate of peeling, and the temperature such that

\[
G = G_0 (1 + f(R,T))
\]

Eqn. 1

In most adhesive systems, the factor \( f \) is much larger than unity, which means that energy dissipated in the bulk has a dominant effect on the fracture strength. The energy is dissipated in the bulk of the adhesive and its magnitude is dependent on the rate of
internal motion of the polymeric chains versus the rate of displacement applied. In
essence, the fracture strength is enhanced by viscous resistance to internal motion [19].
At temperatures much higher than the glass transition temperatures, the internal motions
are rapid, so the polymer strains in a large manner instantaneously and fully reversibly
(rubber-like entropic elasticity) and very less energy is dissipated.

On the other hand, at temperatures much below the glass transition temperature,
the chain motions are essentially “frozen”, such that applied forces are responded to by
bond stretching such that the polymer still responds instantaneously and reversibly (glass-
like enthalpic elasticity) but cannot accommodate large strains and thus fractures in a
brittle manner [20].

In the range near the glass transition temperature, $T_g$, is where maximum energy
is dissipated, the polymer is said to be in a ‘leathery’ regime where it has components of
both viscous liquids and elastic solids. It is ‘viscoelastic’ in nature. The $T_g$ of an adhesive
is one of the most important parameters governing its behavior. Ideally, an adhesive will
always stay in either glassy, leathery, or rubbery region throughout its life-time,
depending upon its application [20].

Rate of applied displacement has, essentially, the same effect as temperature, especi-
ally in linear viscoelastic materials. Depending on the mode of failure (interfacial
or cohesive), rates can different effects. It has been shown that for adhesion of
viscoelastic materials with rigid substrates, at low rates, there is a transition from viscous
like to rubber-like behavior of the adhesive resulting in an increase in fracture strength
[21].
At high rates, there is a transition from rubber-like to glass-like behavior resulting in a sharp decrease in fracture strength. For linear-viscoelastic materials, there is an equivalence between rates and temperature, given by William, Landel, and Ferry using which behavior at different temperatures can be predicted by varying the rates and vice versa. Plasticizers and tackifiers are added to adjust the $T_g$ of the adhesive system. Plasticizers reduce the $T_g$ while tackifiers increase it [21].

1.6 Conclusion

Adhesion and adhesives has a rather old origin. Since then, mankind has been able to employ different plant- and animal-based materials for binding different surfaces. This led to the advent of artificial adhesives synthesized for binding a multitude of surfaces. Synthetic adhesives are a lot simpler than natural adhesives in terms of composition and interactions between those components, which makes them useful for only certain surfaces. To solve this and other daunting problems, mankind has again turned towards nature for inspiration. A framework to understand the basics of adhesion has been laid down in this chapter. Next, we shall discuss a few biological adhesives that are studied by researchers in the hope to find inspiration to develop novel adhesives.
2.1 General Introduction

In Nature, many organisms, ranging across all different length scales and environments use different kinds of adhesive polymers for locomotion, self-defense, and prey capture. It is very interesting to note that the very first glues used and produced by mankind were derived from plant and animal sources [1]. Then biological materials: blood, bones, hair, hides were used to make adhesives. Around the time of the industrial revolution, the first synthetic adhesive was synthesized and there has been tremendous growth of adhesives since then [3]. Today, it is a multi-billion dollar industry. Despite this amazing history, there still are several questions in the field of adhesives that remain unanswered. To solve these questions, mankind has again turned to nature for inspiration. This chapter discusses a couple of the strategies employed by nature to adhere in different circumstances. As will be seen in the chapter, adhesion is a key aspect in the lives of many organisms. Since cell-cell adhesion is a fundamental process in every living organism’s body, it is not an exaggeration to state that there would be no life without adhesion. While there are literally millions of different adhesive systems in this world,
this chapter briefly introduces only a few of them that have gained some attention by researchers who spend their lives trying to understand and, if fortunate enough, mimic these adhesive systems.

Perhaps the biggest dichotomy between natural and artificial adhesive systems is the tremendous complexity observed in the former. There are several different components present which play different roles in the adhesion process, which makes it difficult to understand and characterize the adhesive completely. Most of the mimicry has thus been done on the basis of a very small aspect of a rather big picture. Marine mussels and barnacles are classic examples of this. These organisms can form strong attachments under water to almost everything that floats. Scientists have discovered one of the amino acids present in abundance in their attachment plaques and have incorporated that in synthetic materials to form artificial underwater adhesives [22]. The complete picture is not understood well and is far from replication. Progress is being made rapidly though. Another important characteristic of most natural adhesive is the non-specific nature of adhesion. These adhesives can stick to almost everything from wood, pebbles, leaves, glass and plastic, and that too reversibly. This is one feat that most synthetic adhesives have still not accomplished.

A critical aspect often overlooked by researchers is the entirety of a particular natural adhesive system. The adhesive ligand used in the adhesive would not explain the adhesive in the big picture. The structure of the adhesive at the nano-, micro-, and macro-scales together with the polymer and other components, if any, can describe the why’s
and how’s of a certain system. A few examples of biological adhesive systems are introduced as follows.

2.2 Biofilms

Biofilm, in layman terms, means a colony of microbes. Contrary to popular belief, the microbes can be anything from bacteria to protozoa to fungi to algae to archaea. Some films may have more than one micro-organisms present. Bio-films dwell in warm and humid places so medical devices implanted in the body is a big and easy playground for these films to develop [23]. Understanding the growth of biofilms and developing methods to prevent/stop has gained attention amongst scientists of various disciplines. These films can essentially form on any living or non-living surface, given the right conditions [24].

The growth of these films starts when a free-floating microbe attaches to a surface. Subsequent microbes either attach directly to the surface or to an already attached microbe [25]. The attachment to surface uses van der Waals forces at first, after which structures such as pili are used. Adhesion between microbes, however, is mediated because of extra-cellular polymeric substances which comprise of DNA, proteins, and polysaccharides. The film has mechanical properties similar to slime (viscoelastic fluid). The cohesive strength comes largely from the polysaccharides, in addition to the microbe-microbe adhesion. Proteins function as a primer and facilitate adhesion on different surfaces [26].
Similar chemistry of the adhesive is also observed in fungi [27] and algae [28]. Fungi use a glycoprotein based molecule ‘adhesin’, to make tenacious attachment to even relatively inert surfaces [29] while certain algae locate a suitable surface and bind to the surface by secreting an adhesive that is again glycoprotein based [30].

2.3 Marine mussels

A perfect example to demonstrate the difference between the complexity observed in natural and artificial adhesives is the byssus threads produced by marine mussels. Byssus threads are a multi-component complex system connecting mussel tissue with under-water rocks/ships etc [22, 31]. There is a gradient of stiffness in the byssus threads. As the thread goes towards the rock, there is a soft stem, a rubbery proximal portion, a stiff distal portion and an adhesive plaque [22]. Our interest lies in the adhesive plaque which is composed of a couple of well-characterized proteins, collectively called, mefp for *Mytilus edulis* foot proteins. These proteins are rich in the post-translationally modified amino acid DOPA (Dihydroxyphenylalanine) [32]. As we discussed in the first chapter, fracture energy depends on both interfacial bonds and bulk dissipation [19]. The byssus thread is composed of collagen and silk which provide the necessary bulk properties which the adhesive plaque proteins provide the interfacial bonds [32].

Researchers have used DOPA chemistry to synthesize polymers that can stick under water. DOPA has been incorporated into the branches of different polymers to make adhesives that can stick in fluidic environment [33]. Due to the ability of this
molecule to form different bonds with different surfaces, the polymers synthesized could stick to different surfaces such as metals and plastics. These synthetic polymers have been used in various applications like cell culture, functionalization of different surfaces, development of anti-fouling surfaces [34], which is particularly interesting, and also ironic in a certain way, since marine mussels are one of the biggest sources of bio-fouling and researchers are actively trying to prevent mussel-adhesion to ships to cut down on fuel costs.

2.4 Gastropod

Gastropods use a rather unique adhesive system: adhesive gels [35]. As discussed in the first chapter, most adhesives are applied at a lower viscosity and are later hardened to develop cohesive strength such that the final form of the adhesive consists of either polymer or crosslinked material. Interestingly, the adhesive used by gastropods is a crosslinked material to begin with, containing about 95% water. Similar to the adhesive systems discussed above, the chemistry remains largely same: protein-carbohydrate polymers [36]. However, unlike the above-discussed systems, in addition to large molecular weight polymers, small adhesive polymers are also present in these unique adhesive gels. These small adhesive polymers are shown to be capable of crosslinking other polymers present. The high molecular weight polymers can tangle very easily such that these entanglements can form a transient network of their own. Interestingly, in the
non-adhesive from of the gels produced by these creatures, these small molecular weight polymers were absent indicating that these polymers play a major role in adhesion [37].

2.5 Australian Frogs

As mentioned before, the most prevalent uses of adhesives in nature are locomotion, self-defense, and prey capture. Australian frogs kill two birds with one stone with their unique adhesive strategy. These frogs secrete an adhesive material from their dorsal skin for self-defense and prey-capture [38]. This adhesive consists of an array of compounds: simple and complex aliphatic, aromatic and heterocyclic molecules, as well as a range of small and large peptides. This adhesive hardens rather rapidly as an elastic solid which adheres well even in the moist habitats that these frogs dwell in. One of the demonstrated uses of these adhesives is to jam the jaws and other fatal limbs of the predator so that they are stuck to the skin of the frogs which they later shed and consume [39].

2.6 Geckos

The importance of bulk properties and structure in adhesion cannot be better demonstrated by anything other than the gecko toe system. Gecko toe pads consist of an
extraordinary hierarchy of structure [40] that functions as the adhesive that enables geckos to run on smooth and rough, clean and dirty, dry and wet surfaces, and climb on vertical walls and stick to even inverted surfaces. Since geckos are required to run, the toes must be reversibly adhesive. Also, in order for them to run fast, they must be able to establish and release proper contact within milliseconds. Also, adhesive on the gecko toes cannot weaken because of dirt. All these seemingly contradictory requirements are achieved by geckos by an atypical material and a rather clever structure used beneath its toes. Thousands of micron-sized setae cover its toes. Each one of these setae further split into several nano-sized spatulae. These setae and spatula are made of β-keratin, a rather stiff material [41]. The requirements of an adhesive: to establish intimate contact and resist separation are both satisfied here. Intimate contact is established because these nano-hairs are compliant in bending. However, they are rather stiff in tensile and shear directions so they resist separation [42]. Unlike, any of the adhesives discussed above, these adhesives have been shown to be dry. Recently, however, it has been demonstrated that the toes release phospholipids upon contact [43]. Since they can stick to any surface, van der Waals has been thought to be the predominant adhesive mechanism which is rather interesting since these nano-hairs do not stick to themselves [12]. Adhesion to surfaces weakens in water at short times, but at longer times, it has been shown to adhere strongly to hydrophilic surfaces [42]. Easy release of contact is accomplished by peeling at a higher peeling angle so that adhesion reduces. Inspired by its clever design, a lot of research groups have fabricated a variety of hierarchical surfaces for different purposes [44].
CHAPTER III

SPIDER SILK ADHESION

3.1 Abstract

Spiders employ clever behavioural strategies combined with almost invisible custom-made adhesive silk fibers to spin prey capture webs. The adhesives used in these webs evolved over millions of years into a class of natural materials with outstanding properties. In this chapter, different adhesives used by spiders to capture prey are discussed. Spiders use different strategies to capture their prey, from adhesive webs to adhesive gluey balls to hunting. Different adhesives and strategies used by spiders are discussed in order to give a background and lay the foundation for the research that follows in the subsequent chapters [45].

3.2 Introduction

Bonding different materials together by means of an adhesive may appear mundane to most people. In reality, a great deal of science and technology is involved in this simple
action of bonding. Adhesive manufacturing continues to grow due to the diversity of substrates and the continuous introduction of new chemistry and processes. However, long before human industry, nature evolved many well-designed adhesives for locomotion, defense, and prey capture. As we saw in the previous chapter, geckos use micron-sized hairs as dry reversible adhesives for locomotion [44]. Mussels secrete specialized proteins to stick to rocks under water [33]. Spiders employ multiple kinds of silk fibers in different web-building strategies to capture prey, which is the focus of my doctoral dissertation work [46]. In this chapter, we discuss some fascinating examples of adhesives used by spiders in the hope to stimulate the use of these principles in designing new adhesives. Also, the adhesive systems of spiders that I worked with for my research are introduced.

3.3 Spider Webs

Webs evolved early in the evolutionary history of the world’s 41,000+ species of spiders [47]. Webs provide spiders with the means to trap their food, a place to shelter, and even an arena in which to mate. Webs are assembled from several unique types of silks that function together as integrated units helping to make spiders highly efficient and successful predators. Almost all of the spider families have web-building members [48]. Their web designs and prey-capturing strategies, as shown in Figure 3.1, range from two-dimensional sheets to three-dimensional tangles to the wheel-like orb web [49]. The spider’s web is primarily a trap, mostly for insects. Webs first stop or slow prey and
then transmit the location of the trapped insects to the waiting spiders. This represents a formidable challenge because of the high kinetic energy of prey, especially flying insects, and spiders must react quickly to prey to prevent insects from escaping. This places a premium on the adhesive capabilities of spider silks.

Spiders evolved many interesting and innovative strategies that use silk to capture prey over their ~400 million year history [49]. Almost all of these strategies involved spiders spinning multiple kinds of silk threads ‘custom-made’ for different functions within webs. Given the immense variation in web architecture among spiders, it is no surprise that different types of silks evolved unique sets of material properties and that silk performance can vary immensely across different species [50].

Silkworm silk is an important and high-priced textile commodity used for thousands of years [51]. In contrast, spider silks have yet to be utilized at a large scale despite their desirable qualities because of the difficulty of “farming” spiders. Only rather recently have we begun to realize that spider silks can inspire us to make high-performance synthetic mimics for myriads of applications. Much research is underway to characterize the protein ‘tool-box’ that the spiders use to spin these “intelligent” biomaterial fibers [52] and to express these proteins in more conveniently farmed organisms like goats, bamboo trees, bacteria, and silkworms [53]. The main aim of this review article is to discuss the adhesion of viscid and cribellar capture silks in orb webs. However, the orb is an intermediate “stepping stone” in the evolution of web spinning, facilitating new innovations in silks and webs. Thus, we first introduce a few of the
diverse silks and web spinning strategies of spiders, as shown in Figure 3.1 [47] to provide context.
Figure 3.1| Different kinds of prey capturing strategies. Optimization of spider web architectures on an evolutionary tree of spiders. Adapted from [49]
3.4 Bolas Spider

Bolas spiders (*Mastophora* spp.) are atypical orb-weaving spiders that do not weave a typical orb-web. Instead, they hunt mostly male moths by using a sticky blob at the end of a silk fiber, known as a 'bolas' (*Figure 3.2*). By swinging the bolas at flying male moths or moth flies in their vicinity, these spiders snag their prey much like a fisherman snagging a fish on a hook. The female spiders provide a remarkable example
of aggressive mimicry when they release chemicals similar to the sex attractants that are produced by female moths to attract the male moths [54, 55]. The gluey bolas silk evolved from the viscid silk of normal orb-weavers but overcomes a serious challenge – the scales of moths and butterflies rub off easily allowing these insects to escape most orb webs. The bolas glue in contrast soaks through the scales and adheres to the underlying cuticle of these challenging preys.

Figure 3.3| Ribbon silk. Figure shows an SEM image of the major ampullate silk spun by female L. laeta. The silk is unique in its highly anisotropic shape, compared to the relatively cylindrical silk of most other spiders. Arrows point towards the plaque of adhesion between ribbons. Scale bar is 10µm. It is adapted from [56]
3.5 Brown Recluse Spiders

Famed for their hemolytic venom [57], which can cause necrotic lesions, these spiders (*Loxosceles* spp.) also spin silk retreats underneath objects. These retreats usually consist of two sheets of silk threads with a space between them for the spider and a tangle of loose threads outside the sheets. The lower sheet is in contact with the substrate while the upper sheet, attached to the underside of objects, has a small hole which the spider uses to exit. While the general structure of these webs is characteristic of the behaviors of many groups of “primitive” spiders that mostly lack adhesive silk, the silk produced by brown recluse spiders is noteworthy. These sheets are composed of a maze of ribbon-like, rather than cylindrical, silk anchored to surfaces by thousands of very fine threads (*Figure 3.3*). The silk ribbons adhere very well to each other and the whole network is highly elastic with ribbons capable of extending up to twice their length with low hysteresis [56, 58].

3.6 Black Widow Cobwebs

The cobweb of the black widow spider (*Latrodectus hesperus*) has three dimensional structure comprised of two distinct prey capture surfaces, a catching sheet and supporting threads that can physically entangle flying insects and sticky gum-footed threads that target walking prey. Sticky gum-footed threads are vertical and extend from
Figure 3.4 Cob webs. A schematic of a cob-web. Adapted from [59]

the substrate to the catching sheet of the cob web. They are easily detached from the substratum when disturbed by walking prey. Glue droplets at the bottom of the sticky gum-footed threads adhere to the prey. Typically, one sticky gum-footed thread contains four fibers of silk. During web construction, the spider marks suspension sites on the scaffolding silk prior to laying any gum-foot. This site becomes the top, or vertex, of the gumfoot thread. At the vertex, a cement material serves to mechanically link the scaffolding and sticky gum-footed threads. During web construction, the widow spider spins the first pair of threads from the vertex to the substrate. The spider then attaches the sticky gum-footed thread to the substrate and begins spinning the second pair of threads as the spider crawls back to the vertex. Nearly simultaneously, the lower sticky gum-footed threads are coated with viscid droplets. The viscid droplets extend 0.5 to 2 cm on
Figure 3.5| Cob webs. Cob webs can capturing flying prey as well as walking insects. When a walking insect contacts a sticky gum-footed thread, it quickly detaches from the substrate and yanks the insect into the air. Figure shows a simulation of this using a white mass instead of an actual insect. It is adapted from [60].

the lower portion of the gumfoot (Figure 3.4). The spider also cuts the first pair of threads midway back to the vertex, allowing the final sticky gum-footed thread to be pulled under tension. When detached, the sticky gum-footed threads quickly yank the prey upwards. Small prey becomes suspended helplessly in the air after detaching a single gumfoot thread (Figure 3.5). Larger prey items are captured with several gumfoot threads and by active attack [59, 60]. The attack behavior of widow spiders is also noteworthy because they utilize a liquid that is secreted from a unique, enlarged set of aggregate glands that they fling onto prey. This glue appears to harden rapidly over a several seconds.
Figure 3.6| Orb-web. Figure shows a typical orb-web.
3.7 Orb-weaving spiders

These spiders, like most spiders, produce many types of silk, typically seven, each of which has specific properties that appear to be optimized to perform key functional roles. Dragline silk, produced by the major ampullate glands, makes the spokes (or radii) of the wheel-like orb web (Figure 3.6). The spiders also produce minor ampullate silk to accompany the dragline silk in the web, as well as flagelliform silk that form the core filaments of the orb web’s capture threads. The web threads are anchored to the vegetation and affixed to one another by silk cement originating in the pyriform glands. The eggs are encased in very fine silk filaments from the tubuliform or cylindriform and one type of aciniform gland, while another type of aciniform filament is used for a multitude of other purposes such as strengthening the cement matrix. The orb-weavers used two kinds of capture threads to capture prey: either cribellar silk or viscid silk [61, 62]. Cribellate capture silk is relatively ancient and utilized by many types of web spinning spiders while viscid silk evolved more recently and is used by most modern orb weaving spiders [63]. Understanding the structure, morphology, and adhesive mechanism of both of these kinds of glues is the main goal of this article.
Cribellar silk

The cribellate spiders have a unique silk producing structure called a cribellum just in front of the spinnerets. This broad plate is set firmly in the spider’s abdominal cuticle and is covered with thousands of tiny spigots [64]. This cribellum plate produces swathes of the finest gossamer silk, which, when drawn out by combs on the spider’s legs, come out hackled. Charge is imparted to these drying filaments while combing, causing these nanofibers repel each other and puff out to form a nanoscale, wool-like

Figure 3.7 | Cribellar Silk. a) A completed cribellar thread often forms a series of regularly spaced puffs that are brushed into place by the spider’s legs. The surface of these threads is made up of thousands of nanofibers. These nanofibers can be either non-noded (b) or noded (c). It is adapted from [65].
yarn. The resulting nano-filamentous mesh adheres to, and often totally covers, a pair of much thicker (micron-sized) supporting fibers issuing from spigots on the main spinnerets [66, 67]. Some spiders further reinforce this multi-fiber assembly by imparting crimped, spring-like fibers from yet another set of spigots, which pull up tight by the now rather complex, composite fiber [66]. Hackling cribellum silk is expensive for the spider, in terms of the time it takes to produce and the energy it expends. This is pretty evident by the fact that a cribellate spider moving along and laying a thread in its web is slow and also that its two hind-legs rapidly comb away the nanofibers [61].

A completed cribellar thread often forms a series of regular puffs (Figure 3.7a). Adhesiveness of this thread depends on the density of the nanofibers that form its surface and is modified by the dimensions of the puffs and the manner in which a spider loops and folds a finished thread [68, 69]. There are approximately 3606 species that spin cribellar silk threads. Of these, 11 species produce primitive, cylindrical, non-noded nanofibers and the rests produce nanofibers with regularly spaced nodes [70] (Figure 3.7b and c). A couple of different adhesion mechanisms account for the adhesiveness of these cribellar threads. Indubitably, mechanical interlocking provides one mechanisms for capturing insects as the nanofibers on the thread’s surface snag an insect’s setae and retain them just like how a Velcro™ fastener works [71]. Interestingly, this hierarchical structure also adheres to smooth surfaces such as glass and graphite just like the gecko toe pad. Electrostatic attraction, van der Waals forces and hygroscopic (capillary) forces were all hypothesized to account for cribellar silks’ adhesiveness and tested. Quantifying adhesion of these micron-sized threads involves placing the thread between two legs of a cardboard mount, bringing it in contact with a solid substrate, and then detaching the
thread at a controlled rate such that the force exerted just before pull-off is taken as the force of adhesion [72]. No significant difference was found in the force of adhesion values for different substrates when cribellar threads were adhered to substrates of very similar texture but different dielectric constants, which ruled out electrostatic adhesion [73]. This just left two possible mechanisms to explain adhesion, van der Waals forces and hygroscopic adhesion.

Subsequent studies conducted by Hawthorn and Opell repeated these tests in environments with different humidity to determine the possible role of water for adhesion of cribellar threads. Again, the presence or absence of humidity did not affect the adhesion of primitive cribellar threads (non-noded nanofibers). However, evolutionarily derived cribellar threads (noded nanofibers) adhered better at higher values of humidity. Based on these observations, Hawthorn and Opell concluded that primitive cribellar threads likely use van der Waals forces to adhere to smooth surfaces whereas derived cribellar threads can also employ capillary forces. This hypothesis was then tested by modeling the van der Waal forces and capillary forces as follows.

\[ F_{vdW} = \frac{\Lambda_F}{6D^2} \text{ and } F_C = 4\pi R \lambda_L \cos \theta \]

where the subscripts vdW, C, and L mean van der Waal forces, capillary forces and liquid (water), respectively. \( \Lambda \) is the Hamaker constant, taken to be \( 45 \times 10^{-21} \) J, \( R \) is the radius of the sphere (for noded nanofibers, the radius of a cribellar nanofibers node, and for
cylindrical nanofibers, the radius of a cribellar nanofiber) and $D$ is the distance between the sphere and the substrate where van der Waals forces become significant. $\lambda_L$ is the surface tension of water (76 mJ m$^{-2}$) and $\theta$ is the angle of contact between the water and the substrate. Number of contact points per unit area was determined for both primitive as well as derived cribellar threads and the forces were multiplied with the total number of points in contact. A good agreement was found between the experimental and the theoretical results and hence it was concluded that primitive cribellar threads use van der Waals forces whilst the derived cribellar threads employ hygroscopic forces to accomplish adhesion [65]. Surprisingly, in this model, the stretching of the axial fibers was not taken into account when determining the causes of the adhesion exerted by these threads on flat substrates. Also, it was assumed that all the points of contact were contributing equally to the overall adhesion force exerted by the thread, which was later showed to be incorrect.

![Figure 3.8](image)

**Figure 3.8** Viscid silk. SEM (JEOL) image of a completed viscid capture silk spun by *Larinioides cornutus*.

Viscid Silk

Cribellar nanofibers were replaced in webs by the evolution of aqueous-based, chemically adhesive glue in modern orb-weavers (Araneoidea) [74]. This transition to
aqueous glue is associated with a dramatic increase in diversity of Araneoidea compared with its cribellate sister lineage Deinopoidea and can be attributed to the ‘success’ of glue and the composite viscid silk thread over the cribellar threads [49]. Orb-weavers rely upon a combination of strength and stiffness from the dragline silk and stretchiness of the capture spiral to absorb the kinetic energy of flying insects that impact webs [75]. The adhesiveness of the capture spiral then retains insects long enough to be located and captured by spiders [76]. The strength, stretchiness and stickiness of viscid silk capture threads have a synergistic effect which out performs cribellar threads in capturing prey, and hence the resulting increase in diversity of Araneoidea as compared to Deinopoidea.
Figure 3.9] Viscid silk. This kind of a capture silk is spun using a triad of spigots. Axial silk is spun in the flagelliform (FL) gland after which it is coated with aqueous glue produced in the aggregate (AG) glands as shown in (b). Figure is adapted from [49].

The viscid threads of orb-weaving spiders consist of two soft, but highly extensible axial fibers surrounded by aqueous adhesive glue (Figure 3.8). These threads are produced from triads of spigots that lie on the left and right posterior spinnerets. Each triad is composed of a gland that produces an axial fiber (flagelliform gland), two glands which secrete the glue (aggregate gland), and their respective spigots. The spigot from the fiber gland is arranged between the spigots of the glue glands such that glue and fibers are simultaneously extruded (Figure 3.9) [49]. At first the glue covers the fibers evenly but it soon spontaneously forms into a series of more or less regularly distributed droplets due to Rayleigh instability [48, 77]. A number of studies have chemically characterized the components of these glue droplets. Using NMR, the water soluble fraction of this silk was found to contain a concentrated solution of hygroscopic components related to neurotransmitters like GAB-amide, N-acetyltaurine, choline, betaine, isethionic acid, cysteic acid, lysine, serine, potassium nitrate, potassium dihydrogenphosphate, and pyrrolidone. The water soluble fraction however does not contain any polymer. The concentration of salt present determines the amount of water uptake by this system. Also, the high concentration of salts gives vapor pressure very close to ambient humidity values prevalent at the habitat of these spiders. Also, the compounds do not react electrostatically with the anionic glycoprotein because they are either positively charged organic amines, zwitterions, or anions of very strong acids –
sulphonates. Moreover, these salts do not crystallize over a wide range of vapor pressures unlike salts like NaCl [78].

The polymer fraction of the glue drops was dissolved in trypsin and was analyzed for neutral and amino sugars using Masamune-Sakamoto method and amino acid analyzer, respectively. The results indicated the presence of galactosamine, mannose, galactose, glucosamine, fucose, and glucose [79]. When the cylindrical glue coating applied by the spider breaks into droplets, these compounds assume a ‘drop within a drop’ like morphology. Optical imaging showed that the ‘inner drop’ is fibrous and it was hypothesized that the glycoproteins lie there. Staining the capture silk threads with fluorescent lectin molecules conformed that the N-acetylgalactosamine, and hence the glycoproteins, are present in the ‘inner’ drops. It was hypothesized that the glycoproteins, being the only component in the glue drop with long branches, can act as glue sensu strictu. Sliding the thread between two smooth surfaces resulted in uncoiling and stretching of the fibers in the inner drop after the viscous liquid had dried out, which supported the hypothesis that the glycoproteins do indeed act as the glue [80].

An interesting study investigated the effect of nutrition on the composition and adhesion of capture threads [81] and it was found that spider’s nutritional condition affects the capture thread properties and architectural details of its web. Studies characterized the features of successive webs constructed by unfed spiders that were not allowed to recycle previous webs. The volume of a capture thread’s viscous material (salts + water) and the threads’ inferred stickiness decreases in successive webs, although the capture thread’s extensibility does not change. The lengths of both capture thread and
non-sticky thread decrease at similar rates in successive webs. The decreasing stickiness of capture threads reduces the stickiness per unit capture area. No asymmetry was detected in the spacing of either spiral or radial threads of first and last webs, and no differences were observed in the sizes of viscous droplets in outer and inner spiral turns. This suggested that these spiders assessed their silk resources before they initiated web construction and altered their behavior to produce a highly regular web of an appropriate size for their silk reserves [81].

Attempts were also made to understand the daily and seasonal variation of stickiness of the capture threads [82]. These sticky threads are deposited from the perimeter of the web inward. The hypothesis that depletion of silk reserves during web construction affects the properties of capture threads was tested. The droplet volume (DV) per millimeter thread length was the same in outer and inner capture threads and in early and late season webs. However, the outer threads were stickier than their inner threads and, consequently, had a greater stickiness per DV. Thus, dwindling silk reserves during web construction appeared to reduce the stickiness of threads by changing the composition rather than the volume of their viscous droplets. There were also seasonal declines in both thread stickiness and stickiness per DV, which may result from either the depletion of silk reserves or the reallocation of these resources. Early season webs also had greater stickiness per square centimeter of capture area than late season webs, better equipping these early webs to retain insects [82].

When these threads were exposed to osmic acid, the surface of these threads stained black suggesting the presence of fatty compounds in a ‘superficial’ layer on these threads [83] (the NMR of the water-soluble fraction showed highly saturated fatty acids).
Visual observations of a slice of the cross-sections of these droplets also showed the presence of a dense superficial layer. Based on these observations, a two-phase model was hypothesized for these droplets in which the central dense region was the glycoprotein and the surrounding transparent region is the viscous coat [83].

Figure 3.10 | Structure of the glue droplet. a) Figure shows a schematic of the hypothesized three-phase model of the glue droplets. It is adapted from [84].

However, due to recent visual observations and calculations of the relative sizes of the ‘inner droplet’ and the extent of stretching of the whole droplet when detaching from a surface, it was hypothesized that the glue drops assume a three-phase model instead [84]. These three regions are a small central, opaque anchoring granule, a larger
surrounding, transparent glycoprotein glue region, and a more fluid outer covering that extends onto inter-droplet regions (Figure 3.10). This organization would allow droplets to generate adhesion, elongate under a load, transfer force to the axial fibers, and resist slippage on the axial fibers. For doing these measurements, a capture thread was flattened on a microscope slide a small and the opaque granule, which can usually be seen within each droplet, was thoroughly observed. Calculations showed that granule size is directly related to droplet volume and indicated that granule volume is about 15% of droplet volume. Attempts were made to support the hypothesized adhesive role of granules by establishing an association between the contact surface area and volume of these granules and the stickiness of the viscous threads. However, it was shown that granule size made an insignificant contribution to thread stickiness. Consequently, it was hypothesized that granules serve to anchor larger, surrounding layers of transparent glycoprotein glue to the axial fibers of the thread, thereby equipping droplets to resist slippage on the axial fibers as these droplets generate adhesion, elongate under a load, and transfer force to the axial fibers [84].

Yet, the role of water in the aqueous material and the composition of the capture threads remained to be determined. These questions were answered using solution state NMR experiments on silk with the idea that mobile molecules in solids yield high-resolution spectra [85]. Peaks corresponding to the components of the viscid coating, along with the observation of full Nuclear Overhauser effect indicated that the coating is mobile. These peaks disappeared and reappeared on dehydrating and subsequently rehydrating the webs. When these webs were washed with D₂O and dried, subsequent NMR showed low signals consistent with protein. These signals were later associated
with the glycoproteins. The crucial observation here was that, in the presence of water, the viscid capture silk was partly mobile on the NMR timescale. The intensity of this capture web spectrum is roughly comparable with that of a similar quantity of a small soluble protein and suggests that a large fraction of the web is visible in these spectra and is therefore mobile. Capture fibers therefore act like an elastomer that is well above its glass transition temperature and has very low crystallinity. The loss of NMR signals when the silk dried showed that the water acts as a plasticizer for the silk. The difference in composition of radial silk compared to capture silk is evidenced by its lack of significant NMR signals in water. Water thus plays a huge role in the capture silk elasticity [85].

It is interesting that such a complex system – salts + glycoproteins + stretchy flagelliform silk is spun and recycled almost every day. It was shown that this adhesive system has the potential to work much longer than the spider gives it credit for [86]. This adhesive system normally functions for less than a day before being replaced. Despite their ephemeral nature, it was found that the stickiness of viscous threads persists for much longer. When measured over the course of 7 days, small decreases in the capture silk adhesion were not statistically significant. Capture threads were aged for 8–10 months and re-measured under environmental conditions similar to those under which initial measurements were made. When returned to humidity similar to that under which measurements were initially made, neither the droplet volumes nor the stickiness of aged threads differed significantly from those of newly spun threads. These observations indicate that when viscous threads are protected from contamination, the compounds responsible for their hydrophilic and adhesive properties do not degrade easily [86].
3.8 Adhesion of cribellar and viscid threads

There have been numerous, mostly indirect, studies aimed to understand the adhesion of cribellar and viscid silk threads. Also, the effect of different factors like, web orientation, chemical composition, tension in fibers on their adhesion and the effect of adhesion on the prey-capturing ability have been studied via regression analysis, and based on those studies many hypotheses have been put forth.

Opell discusses the effect of web orientation and chemical composition on prey interception in cribellar and viscid thread-containing orb webs [87]. Cribellar prey capture threads found in primitive, horizontal orb-webs reflect more light, including ultraviolet wavelengths, than viscous threads found in more derived, vertical orb-webs. Low web visibility and vertical orientation are each thought to increase prey interception and may represent key innovations that contributed to the greater diversity of modern, araneoid orb-weaving spiders. He compared prey interception rates of cribellate orb-webs with viscous orb-webs. Sectors of cribellar and viscous threads were placed side by side in frames that were oriented either horizontally or vertically. Both kind of webs intercepted more prey when vertically oriented. In each orientation viscous orb webs intercepted more insects than did cribellar orb webs. Although this is consistent with the greater visibility of cribellar threads, the more closely spaced capture spirals of viscous orb webs may have contributed to this difference. These observations supported the enhanced prey interception of vertically oriented orb-webs, but offer only qualified support for the contributions of lower visibility viscous capture threads [87].
Insect features were also shown to affect the adhesion of the viscid silk threads [76]. Spider orb-webs intercept a broad range of insects and their capture threads must adhere to a range of surface textures. In species of the Araneoidea clade, these capture threads are composed of viscid droplets whose size and spacing differ among species. To determine how droplet profile and insect surface texture interact, the stickiness of viscous threads produced by four species using four insect surfaces, ranging from a smooth beetle elytra to the dorsal surface of a fly abdomen that was covered by widely spaced setae, was measured. The adhesion of threads to these surfaces differed by as much as 3.5-fold within a spider species and 2.1-fold for the same insect surface between spider species. However, most of these differences in stickiness was explained by four variables: the ratio of natural log of droplet volume to setal length, the natural log of droplet volume per mm of thread length, setal surface area, and the area of cuticle not excluded from thread contact by setae. Compared with primitive cribellar capture threads produced by orb weavers of the Deinopoidea clade, viscous threads performed more uniformly over the range of insect surfaces. They also held bug hemelytra, which were densely covered with fine setae, more securely, but held beetle elytra, fly wings and fly abdomens less securely than did viscous threads. Hemelytra may be held more securely because their setae more easily penetrate the viscous boundary layer to establish a greater area of interaction and, after having done so, offer more resistance as they are pulled through this layer. Finely textured surfaces may also have higher effective surface energies and therefore may interact more completely with viscous material [76].

One of the very important material parameter for thread adhesion is thread extensibility [88]. The hypothesis that axial fiber extensibility is crucial for adhesion was
tested by comparing the stickiness of unstretched threads with threads that had been elongated to reduce the extensibility of their axial fibers. As stretching these threads also increased the distance between their droplets, the stickiness of stretched threads with contact plates whose widths were increased in proportion to the degree of thread elongation was measured. Actual thread elongation achieved for each individual’s threads and for differences in the five species’ absolute thread extensibility was then accounted for. Models showed that, as threads were elongated, increasing amounts of potential adhesion were lost to diminished axial fiber extensibility. These models indicate that approximately one-third of an unstretched viscous thread’s stickiness accrues from the adhesive recruitment made possible by axial fiber extensibility [88].

Cribellar thread adhesion has also been studied and has been shown to be very different from viscid thread adhesion. Cribellar capture threads are comprised of thousands of fine silk fibrils that are produced by the spigots of a spider’s cribellum spinning plate and are supported by larger interior axial fibers [89]. One study examined factors that constrain the stickiness of cribellar threads and compared the material efficiency of these threads with that of viscous capture threads produced by members of their sister clade, the Araneoidea. An independent contrast analysis confirmed the direct relationship between cribellar spigot number and cribellar thread stickiness. A model based on this relationship showed that cribellar thread stickiness is achieved at a rapidly decreasing material efficiency, as measured in terms of stickiness per spigot. Another limitation of cribellar thread was documented when the threads of two uloborid species were measured with contact plates of four widths. Unlike that of viscid threads, the stickiness of cribellar threads did not increase as plate width increased, indicating that
only narrow bands along the edges of thread contact contributed to their stickiness. As thread volume increased, the gross material efficiency of cribellar threads decreased much more rapidly than that of viscous threads. However, cribellar threads achieved their stickiness at a much greater gross material efficiency than did viscous threads, making it more challenging to explain the transition from deinopoid to araneoid orb-webs [90].

Viscid thread adhesion behaved differently when plates of increasing widths were used. Results confirmed that glue droplets at the edges of thread contact contribute the greatest adhesion, with each successively interior droplet contributing only 0.70 as much adhesion. Thus, regardless of the size and spacing of a thread’s large primary droplets, little adhesion accrues beyond a span of 20 droplets. From this pattern, an index, effective droplet number (EDN), was computed that describes the total droplet equivalents that contribute to the stickiness of thread spans. EDN makes the greatest positive contribution to thread stickiness, followed by an index of the shape and size of primary droplets, and the volume of small secondary droplets. The proportion of water in droplets makes the single greatest negative contribution to thread stickiness, followed by a thread’s extensibility, and the area of flattened droplets. Although highly significant, this six-variable model failed to convincingly describe the stickiness of six species, a problem resolved when species were assigned to three groups and a separate model was constructed for each. These models place different weights on the variables and, in some cases, reverse or exclude the contribution of a variable. Differences in threads may adapt them to particular habitats, web architectures or prey types, or they may be shaped by a species’ phylogeny or metabolic capabilities [90, 91].
Certain propositions were put forth on how the transition from cribellar silk to viscid silk happened [92]. Evolution of orb-weaving spiders that comprise the Orbiculariae clade involved a transition in the composition of prey capture thread that has been challenging to explain. The primitive cribellar threads spun by members of the Deinopoidea subclade resemble the capture threads of their non-orb-web-weaving ancestors and are formed of thousands of fine, dry, protein cribellar fibrils. In contrast,
the derived viscous capture threads spun by members of the Araneoidea subclade have regularly spaced, aqueous adhesive droplets. When second instar deinopoid spiderlings emerge from egg sacs they are unable to spin cribellar threads, and, therefore, do not construct orb-webs; whereas second instar araneoids spin capture threads and construct orb-webs. In an interesting study, it was hypothesized that if viscous material evolved to enable second instar spiderlings to construct orb-webs, early araneoids may have spun composite cribellar–viscous capture threads. To examine the functional feasibility of such intermediate capture threads, the adhesion of cribellar threads, viscous threads, and combined cribellar–viscous threads was compared (Figure 3.11). The stickiness of these combined threads was greater than that of native cribellar or viscous threads alone. Thus, if early araneoids retained their ability to spin cribellar threads after having evolved glands that produced viscous material, their composite threads could have formed a functional adhesive system that achieved its stickiness at no loss of material economy.

Based on the previous studies, it is clear that differences in the dimensions of the adhesive material and the mechanical properties of the underlying axial fibers suggest that cribellar and viscous capture threads function differently in achieving adhesion values per volume of thread material. These differences include the scale at which a thread interacts with a surface, the efficiency with which adhesive forces are transferred to the thread’s axial fibers, and the ability of a thread span to recruit adhesion from interior regions of contact. Cribellar threads interact via nanofibers that are around 20 nm in diameter. Nanofibers of evolutionarily derived cribellar threads have regularly spaced 35 diameter nodes that establish around 170 contact points per µm². In contrast, viscous threads typically have 30 or fewer droplets per mm and mean droplet dimensions of
10 μm or greater. Thus, cribellar thread generates adhesion at many small, diffuse points of contact, whereas viscous thread generates adhesion at a few large points of contact [90]. Due to the diffuse nature of the contact established by the cribellar threads, the adhesion generated at these points is not effectively summed and transferred to the axial fiber. Viscous thread, on the other hand, generates adhesion using much fewer collinear droplets which effectively transfers this force to the axial fibers. Useful adhesion is generated only at the outer edges of contact of a cribellar thread with a surface, based on the observation that cribellar threads do not exhibit any significant difference in the force values when adhered to plates of different widths. The limited softness and extensibility of cribellar axial fibers could explain this effect. In contrast, the greater extensibility of viscous threads, and of the viscoelastic droplets, produces a highly extensible system capable of transferring more force along the thread’s axial lines, hence recruiting more adhesion by inner droplets and is termed the ‘Suspension bridge mechanism’ [90] (Figure 3.12).

Most of the previous studies studying adhesion investigate whole thread adhesion measurements in which a capture thread is brought into contact with a substrate and then retracted from it such that the force registered right before the contact is released is called the force of adhesion. Intra-species and inter-species comparisons are drawn based on this force of adhesion. However, this force of adhesion not only depends on the adhesive in the system but also depends on the mechanics of the axial thread. Hence, it is not a direct measure of ‘adhesion’ in the system. This major concern is addressed in my doctoral dissertation work. We have developed an experimental and theoretical protocol to separate the two contributions and study just the adhesive part of these composite
threads. The aim of my work is to show the importance of structure and architecture at nano, micro, and macro scales on the adhesion of these spider adhesive systems. At the nano level, we discuss how the viscid silk glue behaves like a viscoelastic solid and that the elasticity helps in enhancing the adhesion of the glue. At the micro level, we discuss how the phase-separated micro-structure of the glue drops facilitates its cohesion and adhesion properties. At the macro-scale, we show why a beads-on-a-string structure is chosen by the spiders for their capture threads and how it enhances adhesion. We also discuss structure of other adhesive systems such as the pyriform silk attachment joints, without which the movement and locomotion of a spider as well as their prey-capture webs would be impossible.
Figure 3.12] Extensibility plays a huge role in adhesion. Figure shows a schematic of the difference in the thread adhesion behavior when a cribellar thread and a viscid thread are detached from a surface. The difference in the axial silk extensibility in both the cases can be attributed partly to this effect. It is adapted from [90].
CHAPTER IV

VISCOELASTIC SOLIDS EXPLAIN SPIDER WEB STICKINESS

4.1 Abstract

Modern orb-weaving spiders have evolved well-designed adhesives to capture preys. This adhesive is laid on a pair of axial silk fibers as micron-sized glue droplets that are composed of an aqueous coat of salts surrounding nodules made of glycoproteins. We measured the adhesive forces required to separate a small microscopic probe after bringing it in contact with a single glue droplet. These forces are highly rate-dependent and are two orders of magnitude higher than the capillary forces. The glycoproteins in the glue droplets behave as a viscoelastic solid and the elasticity is critical in enhancing adhesion caused by the specific adhesive ligands [93]. These results have important implications in mimicking bio-adhesives.

4.2 Introduction

Modern orb-weaving spiders use adhesive silk threads to capture prey. These threads consist of highly evolved intricate composites of viscoelastic axial silk fibers covered by micron-sized glue droplets [62]. These glue droplets are composed of granules of glycoproteins surrounded by viscous aqueous coats of salts that regulate water content [78, 80, 94]. Although the glycoproteins are hypothesized to cause
adhesion, the mechanism is not understood. This is partly due to an inability to probe the
glycoproteins through the viscous coat and partly due to the difficulty in separating the
contribution of the glue droplets from the highly extensible axial silk for the force
required to peel a single thread off a surface [90].

Here, by probing individual glue droplets, we show that the glycoprotein behaves
like a viscoelastic solid – a property that has important consequences in enhancing the
adhesion of these almost invisible threads. At slow extension rates similar to the
movements of already entangled insects, the drops deform like an ideal elastic rubber
band, which is essential in retaining trapped insects long enough to be subdued by the
spider. At rapid extension rates, the adhesive forces are dramatically enhanced due to
high viscous effects, making it easier for the capture silk threads to hold on to fast flying
insects when they initially impact webs. The elasticity of glycoproteins enhances the
overall adhesion of the glue by two orders of magnitude in comparison to capillary forces
of the droplet itself, thus putting to rest the old notion of the adhesive being viscous. The
elegant use of elasticity to enhance adhesive forces also occurs in marine mussels and
therefore suggests a common design principle in the evolution of natural adhesives [31].

4.3 Methods

Single drop pulling: Capture threads from orb webs spun by Lariniodes cornutus
were immobilized on a glass slide. Measurements of adhesion were performed using an
MTS Nano Bionix that measured force to ±1µN. The glass slide was fixed firmly on the
lower clamp while a clean conical glass probe (base diameter = 10 µm) was fixed on the
upper clamp. To measure adhesion, the conical probe was lowered at 1 µm/sec till it made firm contact with a glue drop (the whole assembly was observed with an optical microscope). After 60 seconds, the probe was pulled away from the silk at known rates. The stretching behavior of the glue drop was observed using an optical microscope simultaneously with recording the load-displacement response every 0.01 seconds.

Capillary measurements: Polydimethyl siloxane (PDMS) of viscosity 100 cSt (obtained from Gelest Inc.) was filled in a pan of diameter 8 mm and depth 3 mm which was fixed firmly on the lower clamp of Nano Bionix. A cylindrical glass probe of diameter 90 µm, fixed on the top clamp, was dipped in the PDMS at a rate of 1 µm/sec, held there for 60 seconds, and then retracted at known rates. The load-displacement response was recorded every 0.01 seconds.

Thread pull-off measurements: Individual fibers of capture spiral silk were first collected from the webs spun by Larinioides cornutus and adhered to cardboard mounts across 16 mm gaps. After mounting the sample in the Nano Bionix, we pressed the silk thread onto a 2-mm-wide piece of glass mounted on a small tack. The glass was replaced regularly so that every run was performed on a clean surface. The sample was first lowered until it initially contacted the glass, and then pressed until the force registered 80 µN, to ensure firm contact. Finally, the silk was pulled away from the substrate at known rates. The stickiness was measured directly as the force registered when the silk released from the substrate.

Confocal microscopy: To observe swelling of glue droplets, individual threads were equilibrated at a desired humidity. After equilibration, the threads were pressed onto a
cover slip so that the force registered was 80 µN. The cover slip was covered with another cover slip quickly and the edges were then sealed to maintain the targeted humidity. The images were then taken using an Olympus IX71 with a 100X objective.

4.4 Results

Capture silk threads are immobilized on a glass substrate and a conical glass probe of 10 µm base diameter is brought in contact with the glue droplets as shown in Figure 4.1a and Figure 4.1b. The probe is then retracted at constant speeds (Figure 4.1c) while the force is recorded as a function of distance (Figure 4.1d). After reaching the critical pull-off force, the tip releases contact. The visual image of the glue droplets stretching is shown in Figure 4.1c. The force-distance response is highly dependent on the rate of pull-off. The pull-off forces increase from 60 µN at a rate of 1 µm/s to around 400 µN at 100 µm/sec (Figure 4.1d).

To eliminate that pull-off forces are due to capillary effects, we measure capillary effect-induced pull-off forces by dipping the conical probe in a model polymeric viscous liquid (unentangled polydimethylsiloxane (PDMS), because it has high affinity for glass). These pull-off forces are below the detectable limit of the instrument (1 µN). Therefore, we use a larger diameter probe (90 µm) to measure the capillary effect-induced pull-off forces and then estimate the capillary forces for a 10 µm diameter probe using the Wilhelmy equation ($f_c = \gamma \times \text{perimeter} \times \cos \Theta$). These estimates for PDMS ($\gamma \sim 20$ mN/m) and a liquid of surface tension 40 mN/m (typical values expected for aqueous solutions such as viscous coat) are 0.6 µN and 1.3 µN, respectively (green and black
markers in Figure 4.2a. The pull-off forces exerted by the glue droplets are two to three

Figure 4.1 | Single drop adhesion experiments. Figure a shows the components of the capture thread of the spider Lariniodes cornutus: 1. Viscous coat, 2. Glycoprotein granule, and 3. Axial thread. Scale bar = 20 µm. Recently, it has been suggested that the viscous coat contains transparent layers of glycoproteins [84]. Inset shows a schematic of the single drop pulling experiments wherein single glue drops (green) of a capture thread were stretched using a conical glass probe (blue) while the force responses were recorded. Figure b shows the conical glass probe approaching a single glue drop. Scale bar = 50 µm. Figure c shows stretching of a single glue drop using the conical glass probe. Scale bar = 60 µm. Figure d shows the force responses when single glue drops were stretched at different rates until separation from the glass probe. The curves are plotted as mean ± standard deviation from three measurements each (error bars are in black for all the three cases).
orders higher than the capillary forces, indicating that the primary adhesive in the system is the glycoprotein and not the viscous coat.

Based on the rate-dependence of pull-off forces, the glue could be either a viscoelastic liquid or a viscoelastic solid. If the glue is a viscoelastic liquid, then the glue should behave like a liquid at very low velocities. In contrast, if the glue is a viscoelastic solid, then forces will not relax to zero with time. To test these predictions, we measured the force-relaxation after stretching the glue droplets by 100 µm (Figure 4.2b). For high speeds there is a considerable overshoot; however, with time the forces relax to a constant value that is independent of the rate of displacement. The steady state forces, which are two orders of magnitude higher than those expected for capillary forces, clearly indicate that the glue is neither responding as a viscous liquid nor as a viscoelastic liquid. Instead, the glue exhibits characteristics of a viscoelastic solid. This is consistent with the microscopy images of a glycoprotein granule (and the whole glue drop) swelling in water while retaining its shape at high humidity (Figures 4.3a and b). Furthermore, the elastic response and the absence of terminal flow regions indicate that the glycoproteins are cross-linked.
Figure 4.2 | The adhesive forces are dominated by elasticity rather than capillary forces. Figure a compares the pull-off forces at different stretching rates for glue drops (red markers) with the capillary forces exerted by PDMS melt (Mw. = 6000 Da, Me = 8000 Da, γ = 19.8 mN/m, green markers) and the calculated capillary forces exerted by the viscous coat (γ = 40 mN/m). For PDMS-glass and viscous coat-glass, the contact angle Θ was taken as 0. The values are plotted as mean ± standard deviation from three measurements each. Figure b shows load-relaxation curves in which the glue drops were stretched by 100 µm at three different rates (100µm/s (black), 10µm/s (blue), 1µm/s (red)) after which the load was allowed to relax. Inset shows an enlarged view of the plateau
regions for the three cases. All the measurements were performed close to 25°C at 40% R.H. The curves are plotted as mean ± standard deviation from three measurements each.

Figures 4.3a and b show transmission mode images of glycoprotein granules at 90% R.H. and 0% R.H., respectively. Scale bar = 20µm.

4.5 Discussion

Viscoelasticity of glue drops has far-reaching consequences on the functioning of capture threads in spider webs. A single capture thread is covered with many of these glue drops and the peeling of a capture silk thread from a surface depends on both the glue drops and the viscoelasticity of the silk fibers. Figure 4.4a (blue markers) shows the force required to peel off a capture silk thread from a glass substrate at different rates. These peeling forces are reversible over many cycles (inset in Figure 4.4d). Interestingly, the peeling forces for whole threads depend on the rate, similar to the
results we obtained for the pull-off forces of a single glue droplet. Because the peeling forces depend on the mechanical properties of both silk fibers and the glue, we need to separate the contributions of each component.

Figure 4.4 | Peeling capture silk thread from the surface depends on the peeling rate. (a) The effect of the rate of pull-off (dh/dt) on the force at pull-off (blue markers and black error bars). Values are plotted as mean ± s.d. from 15 measurements each. (b) Adhesion energies at two pull-off rates using the model explained in the text. (c) The stress – strain behaviour of capture spiral thread at the rate of 2 mm s⁻¹ (red) and 0.1 mm s⁻¹ (blue). The curves are plotted as mean ± s.d. from five measurements each. Error bars are in black for both cases. Measurements were taken at 40 % RH close to 25 °C. (d) Repeatability of the force at pull-off of a capture thread of L. cornutus. Measurements were taken close to 25 °C at 40 % RH. The pull-off rate was 2 mm s⁻¹.

The work performed to pull a silk thread off of a surface is consumed in stretching of the axial silk and the energy required to peel glue droplets from the surface.

Figure 4.5 (inset 1) shows a sketch defining the variables used in the energy model. The
total work on the system \( W_T \) is calculated by integrating the product of the force \( f(h) \) times the infinitesimal height change \( dh \) from \( h \) to \( h+dh \).

\[
W_T = \int_{h=0}^{h=\text{at pull-off}} f(h) \, dh
\]  
   
   \text{Equation 1}

The strain energy stored in the thread when it is pulled from its initial position until it separates from the surface, \( U_{\text{strain}} \), is given by the following equation.

\[
U_{\text{strain}} = \left( \int_{\varepsilon=0}^{\varepsilon = \text{at pull-off}} \sigma(\varepsilon) \, d\varepsilon \right) \pi r^2 (L-D)
\]  
   
   \text{Equation 2}

\( \sigma(\varepsilon) \) is the value of stress at strain, \( \varepsilon \) and \( r \) is the radius of the thread. Stress-strain characteristics of the silk thread depend on rate of deformation and are shown in Figure 3b. Subtracting \textbf{Equation 2} from \textbf{Equation 1} gives the energy required to separate the glue drops from the surface, \( U_{\text{glue}} \) (Figure 4.5). The values of \( U_{\text{glue}} \) depend on intermolecular adhesion as well as the energy required to stretch the glue droplets. The contribution of \( U_{\text{glue}} \) and \( U_{\text{strain}} \) are shown in Figure 4.5 for a peeling experiment at a rate of 2 mm/sec. Using this energy model we determine the values of \( U_{\text{glue}} \) for 0.1 and 2 mm/sec peeling rates (Figure 4.4b). Similar to the single drop experiments, the peeling of the silk thread from the surface is also rate-dependent and is strongly coupled with the elasticity of the glue droplets.
Figure 4.5] Energy model. Figure shows the total work (black) done in separating the thread from the surface, strain energy stored in the axial silk (blue), and the energy contribution of the glue drops (green) when the thread went through the consecutive stages shown in inset 2, 3, and 4. The geometry used for the pull-off measurements is shown in the inset Figure 1. Calculation of $U_{\text{strain}}$ and $U_{\text{glue}}$ involved the reasonable approximation that $D \ll L$. In these experiments this condition is satisfied ($0 \text{ mm} \leq D \leq 2 \text{ mm}$ and $L = 16 \text{ mm}$). The final values of $U_{\text{strain}}$ and $U_{\text{glue}}$ do not depend on the condition that $D \ll L$.

We can now compare the work done by the glue droplets obtained from peeling experiments versus single glue droplet measurements. The area under the load-displacement curve (Figure 4.1d) corresponds to the energy required to pull off the glass fiber after probing the glue droplet. Based on the distance between the centers of the two droplets of $\sim 40 \mu\text{m}$, we estimate that there are 42 large glue droplets in a 2 mm length thread. Multiplying the work done by one glue droplet by the total number of drops, we estimate the upper limit of $U_{\text{glue}}$ (1, 1.4, and 3.4 $\mu\text{J}$ at rates of 1, 10, and 100, $\mu\text{m/sec}$, respectively). However, individual glue droplets neither stretch at constant rates nor to
the same extents during peeling experiments. Also, the surface area of the probe used for single drop measurement is larger than the area of contact between single glue droplets and the substrate during a thread peeling experiment. These factors lead us to conclude that energy values determined from the single droplet measurements define the upper limit of $U_{\text{glue}}$. Most importantly, the rate-dependence of $U_{\text{glue}}$ observed in these experiments illustrates that the viscoelastic properties of the glue play an important role in the adhesion of capture silk threads and their ability to capture prey.

In summary, we show here that the glycoproteins in orb weaving spider capture silk use elasticity to enhance their adhesive forces during prey capture. Although there is no direct evidence of the molecular nature of the glue’s elasticity, a recent study demonstrates that one of the glycoproteins in *Nephila clavipes* capture silk has amino acid sequences similar to elastin and flagelliform spider silk and may therefore possess some level of elasticity [52]. These results suggest that one of the strongest bio-adhesives known to mankind evolved an intricate composition that consists not only of glycosylated and chitin adhesive groups [52], but also an elastic backbone. The marine mussels also use elasticity to enhance the adhesive forces of their byssal threads [31]. We conclude that the mechanical properties of the adhesive play a major role in determining adhesion and that designing a successful mimic of a biological adhesive requires understanding both the ligand responsible for adhesion as well as the elasticity of the backbone structure.
5.1 Abstract

Modern orb-weaving spiders coat their prey capture spirals in their webs with viscoelastic glue consisting of a combination of salts, water, and glycoproteins. The complex cocktail of salts is hypothesized primarily to sequester water from the atmosphere, promoting adhesion by keeping the underlying capture fibers soft and highly extensible. Here, we demonstrate that, in addition to sequestering water, salts play a vital role in generating adhesion and swelling of the glycoproteins. Upon removal of the salts by washing with de-ionized water, the glue loses viscoelasticity, collapses into a hard solid, and thus loses all its adhesion, regardless of water content. The collapsed glue does not regain viscoelasticity, nor its pristine shape and size, upon re-introduction of the salt solution. Salts are necessary to swell and soften the glycoproteins and thus act like a ‘tackifier’, like in pressure-sensitive adhesives, promoting adhesion even in dry silk. Our finding provides a crucial link to understand the evolutionary transition from dry (cribellar silk threads) to viscid capture threads that is associated with a 95% increase in
the diversity of orb spiders. It also suggests a simple mechanism to impart adhesion to materials of desirable bulk properties without altering their surface characteristics – through the addition of salts.

5.2 Introduction

Spiders combine clever behavioural strategies with almost invisible, custom-made, adhesive capture silk threads to spin prey-capture webs [49]. Capture silk threads spun by modern orb-weaving spiders are intricate composites of viscoelastic axial silk fibers covered by micron-sized glue droplets [62]. These glue droplets consist of low molecular weight organic and inorganic compounds [78], in a viscous aqueous solution, that are hypothesized to surround the polymeric glycoproteins [94] [80], which function as the actual adhesive [93]. NMR on the wash of several orb-webs reveals that the aqueous solution is highly concentrated with choline, betaine, isethionic acid, N-acetyltaurine, GABamide, inorganic salts like KH$_2$PO$_4$, KNO$_3$, as well as glycine and highly saturated fatty acids [78] (hereafter referred to as ‘salts’, collectively). These salts are, determined from solution state NMR measurements on whole orb-webs, hypothesized primarily to sequester water from the atmosphere thereby plasticizing axial silk fibers [85]. However, there are no studies describing the role of salts in the adhesion of the capture silk threads.

Here, we study the effect of salts on the adhesion of spider capture threads. We compare the adhesion of pristine capture silk threads and washed capture silk threads (salts are removed upon washing), under different conditions and show that the salts in the aqueous solution play a crucial role in generating adhesion by solvating the
glycoproteins themselves. In the presence of these salts, the glycoproteins become solvated, even when dry, allowing the glycoproteins to establish contact with a surface and adhere to it. Upon removal of these salts, the glycoproteins lose viscoelasticity, collapse into a solid-like block, fail to establish proper contact with a surface and hence do not adhere to it. The salts thus act like a ‘tackifier’ by imparting viscoelasticity to the glycoproteins. Early spider webs used cribellate silk for adhesion, a dry capture silk which depends upon van der Waals forces generated by the tremendous surface area of its surrounding nanofibrils. More than 150 million years ago, orb spiders transitioned from dry adhesive cribellar threads to modern viscid glue, leading to the explosive increase in species diversity of araneoid orb weaving spiders [49]. Our finding that the complex cocktail of salts in viscid glue function in enhancing adhesion, even when dry, provides the first direct evidence for how this remarkable transition in bioadhesives might have occurred.

5.3 Methods

Adhesion measurements: To measure adhesion, capture threads from the webs spun by Argiope trifasciata were suspended across 16 mm gaps in cardboard mounts at their innate tension. Experiments were performed using an MTS Nano Bionix that measured force to ±1µN. A clean glass plate of width 2 mm was fixed on a tack attached to the force sensor while the cardboard mount with silk on it was fixed on the upper clamp. The silk was lowered at a known rate until the thread made firm contact with the glass plate.
(force of 10 µN). After 60 seconds, the silk was pulled away from the glass plate at known rates while the load-displacement behavior was recorded every 0.001 seconds. The force just before the silk thread released contact with the glass plate was recorded as the pull-off force. Force of adhesion data were plotted as mean ± standard deviation from 10 measurements each. The force of adhesion as well as the tensile characteristics did not change upon sonicating pristine silk threads for 1 hour.

Removal of viscous coat: To remove the viscous coat, capture threads, mounted across cardboard gaps, were immersed in de-ionized water and sonicated for 1 hour. These threads were then stored in a desiccator (P2O5). Finally, the threads were equilibrated at desired values of humidity before measuring adhesion.

Tensile measurements: For finding out the energy of adhesion using the energy model developed by us, tensile strain energy, stored in the capture threads before they release contact with the glass plate during adhesion measurements, is to be determined. Capture threads, mounted across cardboard gaps of 16 mm, were clamped on the Nano Bionix such that the ends of the threads were fixed on upper and lower clamps. The tensile tests were performed at rates similar to what the thread experiences during adhesion measurements to account for any viscoelastic effects.

Re-introduction of salts: In the first approach, aqueous solutions containing one, two, three, or all four of choline, glycine betaine, N-acetyltaurine, and isethionic acid of the correct concentrations were prepared and put on a hydrophobic surface (Contact angle 110°). Washed silk thread mounted on a cardboard piece was immersed in that solution
for three hours followed by sonicating in the same solution for another hour. All salts were obtained from Sigma Aldrich.

In the second approach, a water drop suspended from a pipette tip was traversed along three pristine silk threads to collect their salts. The same water drop was placed on a hydrophobic surface and washed capture threads mounted on a cardboard piece were immersed in it for three hours followed by sonicating in the same solution for another hour.

In the third approach, a pristine thread, equilibrated at 100% R.H was laid on a glass plate, very close to which a washed thread was laid, thus forming a composite thread. The composite threads were exposed to different values of humidity and were also sonicated for 1 hour at room humidity (~40% R.H.).

5.4 Results

Capture silk threads mounted across cardboard gaps are brought in contact with a 2 mm wide glass surface, and then retracted from it at controlled rates while the force-displacement response is recorded. The glue droplets on these threads contain a well-characterized [78] cocktail of salts, identified by analysing the water-soluble fraction of whole spider orb-webs. Because the salts are water-soluble, they can be easily removed by washing capture threads with de-ionized water. No polymers (glycoproteins), however, are removed upon washing, as confirmed by the carbohydrate signals in NMR
even after rigorous washing of whole orb-webs [85], and the absence of polymers in the wash of several orb-webs [78]. Pristine capture thread (hereafter referred to as ‘P-thread’) adheres two orders of magnitude more than washed thread (W-thread) (Figures 5.1A and 1B). Moreover, the droplets on the W-thread collapse into hard solid blocks that do not establish proper contact area and do not adhere to surfaces. W-threads fail to adhere even after sliding them on the glass substrate to preload them, a routine technique for synthetic and natural adhesives, like the gecko toes, that improves their contact with surfaces.

Figure 5.1| Effect of Salts on the force of adhesion of capture threads. Figures A and B show the adhesion values for pristine and washed capture threads under different conditions. Notice the difference in the units for both the figures. P and W indicate pristine and washed capture threads, respectively. Inset in Figure A shows the set up used to measure the force of adhesion of single capture threads with a 2 mm wide clean glass plate. The adhesion forces for the washed threads (W) are usually two orders of magnitude less than the pristine threads (P). The difference in the stickiness units between figures A and B has been emphasized using dotted lines. Since the results shown here are pair-wise comparisons, the difference in adhesion values cannot be due to difference in the salt composition of the glue droplets.

If lack of adhesion in the W-thread is due to deficiency of water, rather than salts, in the glue droplets then P- and W-threads should show the same adhesion when they are
completely desiccated ($P_0$ and $W_0$, respectively). Also, a wet $W$-thread should adhere as much as a $P$-thread equilibrated at 100 % R.H. To test this hypothesis, adhesion was compared between a $W$-thread to which water was introduced externally ($W_{\text{wet}}$) and a $P$-thread equilibrated at 100 % R.H. ($P_{100}$). Furthermore, this comparison was juxtaposed with the adhesion of a $W$-thread equilibrated at 100% R.H ($W_{100}$). $P_{100}$ adheres with two orders of magnitude higher forces than $W_{\text{wet}}$ and $W_{100}$ (Figures 5.1A and B). The small difference between the adhesion of $W_{100}$ and $W_{\text{wet}}$ is due simply to excess water disrupting van der Waals and hydrogen bonding between $W_{\text{wet}}$ and the substrate. The adhesion is much lower for both $P$ and $W$ threads when desiccated; however, $P_0$ still adheres with forces that are three times than $W_0$ due to the presence of salts (Figures 5.1A). While we demonstrate that salts alone induce adhesion, the salts clearly act synergistically with water, as shown by dramatic two-orders-of-magnitude increase in adhesion at 100% R.H when salts are present in $P$ thread compared to the more modest three-fold increase adhesion when salt is present at zero humidity, in comparison to $W$ threads. Water alone does not facilitate adhesion because $W_{\text{wet}} < W_{100} < \leq P_{100}$, whereas salts alone are capable of promoting adhesion, although not as strongly as in the presence of water ($P_{100}/P_0 \approx 10^{2}/3$).

An energy model, developed earlier by us [93], is used to determine whether the differences in force values are due to changes in the adhesion of glue drops under different conditions or due to variation in the tensile properties of the axial silk fibers. This model employs the principle of energy conservation and postulates that the work performed to pull a silk thread off a surface is consumed in stretching of the axial silk and the energy required in peeling the glue droplets from the surface. Inset in Figure 5.1A
defines the variables used in the energy model. The total work on the system \( W_T \) is calculated by integrating the product of the force \( f(h) \) times the infinitesimal height change \( dh \) from \( h \) to \( h + dh \).

\[
W_T = \int_{h=0}^{h=h_f} f(h)dh
\]

Equation 1

The strain energy stored in the thread when it is pulled from its initial position until it separates from the surface, \( U_{\text{strain}} \), is given by the following equation:

\[
U_{\text{strain}} = (\int_{\varepsilon=0}^{\varepsilon=\varepsilon_f} \sigma(\varepsilon) d\varepsilon) \pi r^2 (L-D)
\]

Equation 2

\( \sigma(\varepsilon) \) is the stress in a thread of radius \( r \) at a strain of \( \varepsilon \). Subtracting Equation 2 from Equation 1 gives the energy required to separate the glue drops from the surface, \( U_{\text{glue}} \). The values of \( U_{\text{glue}} \) depend on intermolecular adhesion, as well as on the energy required to stretch the glue droplets.
Figure 5.2 | Effect of salts on the energy of adhesion of the glue drops. Figure 5.2A shows the energy of adhesion of the glue drops of W-threads under different conditions, calculated using the energy model developed earlier by us. Energy of adhesion of glue drops of W-threads is independent of humidity even though the forces of adhesion increase with humidity (Figure 5.1A), implying that the difference in forces of W-threads at different humidity is due to the axial silk fibers becoming softer and more extensible with humidity. Figure 5.2B shows the energy of adhesion of P- and W-threads under different conditions. The huge difference between the energies of P- and W-threads for the same conditions indicates that the difference in the force of adhesion for these threads is due to the difference in the adhesion characteristics of the glue drops and not just due to the difference in the tensile characteristics of the P- and W-axial threads. Interestingly, P- and W-threads share very similar tensile mechanics which vary in the same fashion with humidity and rate of pulling. The difference in the energy units between figures A and B has been emphasized using the dotted lines.

Using this energy model, the $U_{\text{glue}}$ values for $W_0$ and $W_{100}$ are equal (Figure 5.2A) implying that the difference in their total adhesion is due primarily to the axial silk fibers becoming softer and more extensible with increasing humidity, rather than a change in the adhesion of individual glue droplets. This observation is supported by optically imaging W threads in air at 0% R.H., 100% R.H., and under water, which show no differences in the sizes of glue drops (glycoprotein blocks) (Figures 5.3C and 3D). It also indicates that the glycoproteins, post washing, do not interact with water because water does not infiltrate its collapsed structure. In comparison, the glue drops of the P-thread expand with increases in humidity (Figures 5.3A and 3B). The irregular shape of the drops after washing, as opposed to their regular (ellipsoidal) shape prior to washing, also indicates that the glycoproteins harden when salts are removed.

Further investigation using the energy model confirms that the two-orders-of-magnitude force difference between $P_{100}$ and $W_{100}$ is due to difference in the adhesion of their glue drops rather than difference in their axial silk’s tensile characteristics because
the $U_{\text{glue}}$ values are two orders of magnitude higher for the P thread (0.31 µJ and 0.004 µJ, respectively) (Figures 5.2A and B). Also, the significant difference in the $U_{\text{glue}}$ values of $P_0$ and $W_0$ (Figure 5.2B) emphasizes the higher adhesion exhibited by pristine glue.

Figure 5.3| Effect of salts on the humidity-response of the glue drops. Figures A and B show optical images of the single glue drops of $P_0$ and $P_{100}$, respectively, and figures C and D show single glue drops of washed capture threads in air (equilibrated at 0% R.H.) and under water, i.e. $W_0$ and $W_{\text{wet}}$, respectively. Pristine glue drops swell when exposed to humidity while washed glue drops remain unaffected even when submerged in water. Scale bar for every image is 50 µm. All measurements were performed at ~25°C.

droplets compared to washed glue droplets, even in the absence of water. These results, combined with the optical imaging, clearly show that the difference in the adhering capabilities of the P- and W-threads is due to the salt solution solvating the glycoproteins and not simply deficiency of water.
Re-introduction of salts

Optical imaging (Figures 5.3C and 3D) as well as the identical $U_{\text{glue}}$ values of $W_0$, $W_{40}$, and $W_{100}$ indicate that the collapsed glycoprotein structure does not interact with water at all. Since the hygroscopic salts facilitate and mediate the interactions between glycoproteins and water, re-introducing salts in the system should, in theory, restore the pristine size, shape, and adhesion of these glue droplets. To test this hypothesis, three approaches were followed.

In the first approach (Figures 5.4A, B, and C), washed silk threads were immersed and sonicated in an aqueous solution of salts containing N-acetyltaurine, choline and glycine betaine. We have used the same concentration of salts reported in the literature [78]. Despite the tremendous variation in the composition and concentration of salts among individuals, among populations, and with the transfer of spiders to laboratory conditions, N-acetyltaurine, choline and glycine betaine are invariably found in the glue drops, and are hence used in this approach. Isethionic acid was also used since it is a major component in the pristine silk thread salt solution [78]. To test the effect of individual as well as combinations of these four compounds, aqueous solutions containing one, two, three, or all of these four salts were prepared and washed threads were immersed /sonicated in those solutions (Figure 5.4B). No change in the sizes and shapes of the glue drops was observed (Figure 5.4C).

It is possible that the glue droplets did not recover due to an unidentified water-soluble salt that is present in the pristine silk thread, but not among the four salts that we used in the re-introduction experiments. To test this, we collected the viscid silk glue salts
by washing the pristine glue droplets using a small water droplet (Figure 5.4D) (second approach). Washed silk was then immersed and sonicated in this water droplet containing the salts (Figure 5.4E). Again, no change in the size and shape of the glue drops was observed (Figure 5.4F).

Figure 5.4|Re-introduction of Salts. Figures A and B show a washed silk thread and the washed silk thread immersed in a salt solution consisting of choline, glycine betaine, and N-acetyltaurine, and isethionic acid respectively. No changes in the size and shape of the washed silk were observed upon removing the thread from the salt solution after three hours of adding the salt back (Figure C). Figure D shows a droplet suspended from a pipette tip collecting salts from a pristine silk thread. 1, 2, and 3 in Figure D show a pristine silk thread, water droplet, and the pipette tip. Upon immersing a washed thread in the same water drop (Figure E), no changes in size and shape of the glue droplets were observed (Figure F). Figure G shows a washed silk thread which was overlaid onto a
pristine silk thread to form a composite thread (Figure H). 1, 2, and 3 in Figure H show a washed glue drop, a pristine silk thread, and the glue of the pristine silk thread, respectively. Upon separating the washed thread from the composite thread, no changes were observed in the washed glue droplets (Figure I). Scale bar = 25 µm.

The water droplet used to collect the salt from the pristine silk thread was much bigger than native glue droplets. This leads to ambiguity regarding the concentration of the salt re-introduced in the glue droplets. It is possible that both concentration and content of the salt solution are important in re-swelling the glue droplets. To maintain the conditions of re-swelling as close to the pristine glue drops, we developed a third approach where the washed thread is brought in contact directly with the pristine glue drops. Figure 5.4G shows the washed glue droplet on a glass slide. A pristine silk thread was overlaid on the washed silk thread as shown in Figure 5.4H. Figure 5.4I shows the washed thread removed after contacting the pristine thread for a period of three hours. Upon separating the washed capture silk, there was no change in the size and shape of the glue drops. Together, these three approaches show that the collapse of the glue droplets upon washing is irreversible and the structure does not recover.

5.5 Discussion

Glycoproteins and salts are present in an aqueous dope in the aggregate silk glands of modern orb-weaving spiders. Spiders coat flagelliform silk fibers with this dope to provide adhesion when spinning the capture spirals of their orb webs. Rayleigh instability causes the initially homogeneous glue coating to self-organize into an array of
micron-sized droplets. These droplets then develop a phase-separated morphology consisting of a dense glycoprotein core surrounded by a sparse and translucent shell [80]. Recently, it was hypothesized that the shell includes a second layer of glycoproteins that is in turn surrounded by a fluid covering consisting of the aqueous salt solution [84]. Based on our experimental results and observations, we hypothesize that the salts are also present in the bulk of the glycoprotein glue itself, rather than simply as a ‘coating’, as was hypothesized before [84]. The salts in the glue droplet play an important role in solvating the glycoproteins and increasing the adhesion and contact with the surface. The hygroscopic nature of the salts helps in swelling the glycoproteins at high values of humidity.

The bulk salts thus act like a ‘tackifier’, like that used in pressure-sensitive adhesives. This explains the irregular shapes assumed by the glycoprotein blocks upon removal of the salts. Negligible adhesion exhibited by the washed glue drops is primarily due to loss of viscoelasticity and not due to the minimal contact area established, since it has been shown that adhesion caused by specific adhesive ligands in the glue drops is at least hundred times weaker than that caused by the viscoelastic glycoprotein bulk [93, 95].

Pristine glue acts like a viscoelastic solid, which indicates presence of crosslinking [93]. This crosslinking and the phase-separation of the glycoproteins from the aqueous shell obviously take place after the spider has coated the flagelliform silk fibers with the aqueous glue-salt dope from its aggregate glands. We hypothesize that the glycoproteins behave like zwitterionic polyampholytes, which is the reason salts are
required to solvate the glycoproteins (‘salting in’) inside the aggregate glands. Spiders likely desiccate the glue-salt dope before coating the flagelliform silk fibers [96] which results in a critical salt concentration that triggers the crosslinking and hence phase-separation of the glycoprotein molecules. This critical salt concentration also explains why capture silk threads spun at extremely low or high humidity have irregular glycoprotein cores [80] (since extremes of humidity alter the salt concentration when capture threads are spun). Furthermore, upon removal of salts, the crosslinked glycoproteins collapse due to intra-and inter-molecular interactions, now possible owing to the absence of the screening effect of the salts, thus forming a hard solid block. However, unlike typical synthetic crosslinked polyampholytes, re-introduction of water and salts in a washed silk thread does not re-solvate and re-swell the glue droplets. We suggest that interactions between the functional groups on the glycoprotein molecules, in the absence of the charge-screening effect of salts, results in excessive bond formations which preclude re-solvation and re-swelling of glycoproteins. Such a phenomenon has been observed elsewhere too [97]. Loss of adhesion upon removal of salts is thus irreversible in the time-scale of our measurements.

The irreversible response of the glue droplets upon re-addition of salts is intriguing considering that the spider webs often experience heavy rain and this should affect the ability of spiders to capture prey if the salt is washed away from the glue droplets. Although systematic studies measuring adhesion before and after rain are lacking, some qualitative studies suggest that the capture silk is less sticky after the rains. However, such considerations are likely trivial because most orb web spiders remove their webs during rains [98]. This behavior is likely in response to the physical damage
caused by rain droplets to the capture but also allows spiders to recover the salts before they are washed away. It is particularly noteworthy that silk recycling does not occur in most web spinning spiders and is largely confined to orb web spiders that produce viscid capture silk.

Viscid silk evolved at least 150 million years ago in the ancestor of araneoid spiders and is now used by over 95% of the world’s ~4500 species of orb spiders [49]. The ancestor of these spiders coated capture threads in its orb web with a sheath of dry adhesive cribellate fibrils that adhere primarily through van der Waals forces [65]. Cribellate capture threads lose adhesion when wetted because of clumping of the fibrils [99], while the viscid silk of araneoid spiders functions poorly when dry [100]. Thus, while phylogenetic evidence strongly demonstrates the homology of these two types of orb webs [49, 101], there is no clear hypothesis for how spiders transitioned from one type of adhesive silk to the other. Our data provide the first plausible hypothesis for how this evolutionary transition in bioadhesives might have occurred. Initially, salts were incorporated into cribellate threads because the salts facilitate adhesion even in dry silk. Cribellate silk represents the earliest known spider adhesive and, while the van der Waals-based adhesion of cribellate fibrils is independent of humidity, the silk produced by cribellate orb web spiders has a noded morphology that makes it responsive to humidity because its microstructure allows for additional hygroscopic adhesion [65]. The hygroscopic nature of the salts would facilitate this added adhesion and we speculate that even slight increases in the presence of salt set up a tipping point that favors the origin of the large liquid glue droplets and glycoproteins of viscid silk – an adhesive that is superior in both stickiness and material economy to ancestral cribellate adhesives [101].
To summarize, our results on the capture spiral threads spun by modern orb-weaving spiders show that the viscous aqueous solution of low-molecular-weight salts plays a vital role in promoting adhesion of the glue drops in addition to keeping the underlying axial silk fibers soft and extensible. We report that the glue loses all its adhesion upon removal of the salts, regardless of water content, because the salts are necessary to solvate, swell, and soften the glycoproteins. Energy analysis and optical imaging results confirm that, without these salts, equilibrating the threads at higher humidity, or even immersing them in water has no effect on the adhesion of the glue droplets. In stark contrast, when salts are present, humidity has a huge impact on the adhesion of the glue drops. Also, even in the absence of water, a capture thread sticks better when salts are present. Re-introduction of salts to washed silk threads does not restore the structure of the glycoproteins and the adhesion of the washed glue droplets. We hypothesize that, in contrast to previous results, the complex cocktail of salts is an integral part of the glycoprotein glue bulk which, in essence, solvates and softens the glycoproteins in the glue drops, imparts them adhesion, and acts like a medium through which the glycoproteins interact with water. Using small molecules to promote adhesion is a strategy exploited in physical and biological adhesives alike. From HEMA-based solutions in dentin adhesion [102] to terpene-based tackifiers for pressure-sensitive adhesives [103], employing small molecules are an easy and inexpensive way to impart adhesion to materials of desirable bulk properties without altering their surface characteristics – in the case of orb web spiders helping to spur the evolutionary diversification of these iconic predators of flying insects.
6.1 Abstract

We compare the prey capture glues produced by orb-weaving spiders (viscid glue) and their evolutionary descendants, the cobweb-weaving spiders (gumfoot glue). These glues are produced in homologous glands but exhibit contrasting structure, properties and response to changing humidity. Individual glue droplet stretching measurements indicate that the gumfoot glue behaves like a viscoelastic liquid in contrast to the viscid glue, which behaves like a viscoelastic solid. Moreover, the gumfoot glue is largely humidity-resistant – elasticity and adhesion are constant across variation in humidity and there is weak volume-dependence. Viscid glue, however, is highly humidity-sensitive. The glue expands an order of magnitude and demonstrates a monotonous reduction in elasticity under increased humidity, while glue adhesion optimizes at intermediate levels of humidity. We suggest that observed differences are
due to different ‘tackifiers’ used in these systems. These results shall inspire future efforts in fabricating stimuli-resistant and stimuli-sensitive materials [104].

6.2 Introduction

Smart materials and devices that can change dimension, properties, and function in response to external stimuli are a current focus of research in both materials and biological sciences. On the other hand, materials that resist particular stimuli are also actively pursued for their own unique applications. Nature contains a myriad of biomaterials that respond differently to external stimuli, ranging across both extremes, and that are often the source of inspiration for developing next-generation materials. The opening and closing of pine cones [105], the rapid and reversible stiffening of connective tissue in echinoderms [106], the coiling and uncoiling of wheat awns [107], and the reversible color change in the feathers of tree swallows [108] are just a few examples of functionally responsive biomaterials, while self-cleaning lotus leaves [109], water-repelling Australian sands [110], and hydrophobic water-strider legs [111] are examples of biomaterials whose function depends upon a lack of responsiveness to key stimuli. These materials and phenomena are just a few examples of the critical role that responsiveness to external stimuli per se plays in the functional adaptation of biological systems. However, evolution itself provides a powerful tool to move biomimetic research beyond simply exploiting individual materials in nature toward understanding the key elements that control environmental responsiveness of biomaterials.
Spider major ampullate (dragline) silk dramatically increases softness and extensibility under increasing humidity [100]. Relatively less is understood about the humidity-responsiveness of spider prey capture glues. Both orb web and cobweb spiders use adhesive silk threads to capture prey that are coated with glue from evolutionarily homologous aggregate glands. However, cobweb spiders evolved from an ancient orb web ancestor in the early Cretaceous [49]. The two lineages of spiders now employ silk glues in completely different webs with very different roles to play in capturing prey, providing an “evolutionary experiment” for investigating changes in the properties and humidity-responses of biological glues during transitions in ecological function. The viscid capture spirals spun by orb web spiders are intricate composites of a core pair of viscoelastic flagelliform axial silk fibers covered by micron-size glue droplets [62]. In contrast, the adhesive capture threads spun by cobweb spiders, gumfoot silk, consist of much larger glue droplets covering two pairs of stiffer major ampullate silk fibers [59]. Viscid silk glue is a complex assembly of glycoproteins [80] that behave like viscoelastic solids [93], and an aqueous solution of low molecular weight hygroscopic salts that regulate water content in the drop [78] and keep the glycoproteins soft and tacky to maintain the stickiness in variable humidity environments, as shown in Chapter 5. Viscid silk functions primarily to retain insects, while the web as a whole dissipates their flight energy [112]. Although the mechanical behaviour of gumfoot silk glue remains unknown, the major ampullate dragline silk upon which the glue is laid is orders of magnitude stiffer than orb web spider flagelliform silk [59] and the glue itself contains two novel peptides with metal-binding properties [113]. Gumfoot threads also target walking, rather
than flying, insects [114]. Individual gumfoot threads act as spring-loaded traps, with the tension in the cobweb literally pulling small pedestrian insects off of the ground [60].

Here, by probing individual glue droplets we show that the gumfoot silk glue droplets behave like viscoelastic liquids in contrast to the viscoelastic solid behavior of viscid silk glue droplets. The viscid silk glue droplets exhibit maximum stickiness at intermediate humidity (40% - 60% R.H). At low R.H., the droplets are very stiff and dense, fail to establish proper contact, and hence adhere less. At higher R.H., lubrication caused by water and low elasticity reduces the adhesion, even though the droplets are softer and spread much better on the surface. Excess water disrupts hydrogen bonding, reduces electrostatic interactions (glycoproteins are negatively charged), and over lubricates, all reducing adhesion. On the other hand, the adhesion of gumfoot silk glue droplets is humidity-resistant. The behaviors of these two glues are in stark contrast to other bioadhesives, such as the monotonous increase in adhesion with humidity of gecko toes [42] and of the cribellar silk [65] produced by orb-weaving Uloboridae spiders. The evolutionary transition in humidity responsiveness of spider silk glue likely reflects functional adaptations to the silks’ new and divergent roles in the webs spun by the orb web spiders and their evolutionary descendants, the cobweb spiders.

6.3 Methods

Single drop pull-off and load-relaxation measurements: Viscid silk threads from orb webs spun by the furrowed orb-weaver Larinioides cornutus and gumfoot silk from
the cobwebs spun by the western black widow Latrodectus hesperus were equilibrated at the desired humidity and immobilized on a glass slide. Measurements of adhesion were performed using an MTS Nano Bionix that measured force to ±1µN. The glass slide was fixed firmly on the lower clamp while a clean conical glass probe (base diameter = 10 µm) was fixed on the upper clamp. To measure adhesion at different values of R.H., the conical probe was lowered at 1 µm/sec onto the droplet till the force registered was 3 µN (the whole assembly was observed with an optical microscope). After 60 seconds, the probe was pulled away from the silk at known rates. The stretching behavior of the glue drop was observed using an optical microscope simultaneously with recording the load-displacement response every 0.01 seconds.

For the load-relaxation measurements, the conical probe was lowered at 1 µm/sec onto the droplet till the probe went the same depth into the droplet for all different values of R.H. After 60 seconds, the probe was pulled away from the silk at known rates such that the drop in contact is stretched by a constant length for and the load was allowed to relax after this. All single drop measurements were conducted close to 25°C.

Thread pull-off measurements: Individual fibers of capture spiral silk were first collected from the webs spun by Larinioides cornutus and adhered to cardboard mounts across 16 mm gaps. After mounting the sample in the Nano Bionix and letting it equilibrate at the desired humidity, we pressed the silk thread onto a 2-mm-wide piece of glass mounted on a small tack. The glass was replaced regularly so that every run was performed on a clean surface. The sample was first lowered until it initially contacted the glass, and then pressed until the force registered 80 µN, to ensure firm contact. Finally,
the silk was pulled away from the substrate at known rates. The stickiness was measured directly as the force registered when the silk released from the substrate.

Stress-Strain measurements: Individual fibers of capture spiral silk were first collected from the webs spun by Larinioides cornutus and adhered to cardboard mounts across 16 mm gaps. After mounting the sample in the Nano Bionix and letting it equilibrate at the desired humidity, the threads were stretched such that the rate of stretching is similar to what it experiences during thread pull-off measurements. For the individual drop force measurements and whole thread measurements, the samples were held at each humidity values for around 5 hours, before starting the measurements (The chamber for these measurements has an inlet through which a mix of nitrogen and water vapor enters). The reason for choosing 5 hours is because the rate of volume change of glue droplets after 5 hours becomes negligible.

Drop volume measurements: Individual silk threads from the webs spun by Lariniodes cornutus and Latrodectus hesperus were mounted on a cardboard piece across a 16 mm gap and were equilibrated in a desiccator (0% R.H.) for 24 hours after which they were placed in a humidifier (100% R.H) and images were taken at t = 0, 30, 60, 90, 120, and 150 minutes using an optical microscope at 100X magnification. The chamber used to humidify the samples is just like a desiccator, except that, instead of the P2O5 pellets, there is water.

Polyethylene oxide (PEO) measurements: PEO/Water solutions of concentrations 13.7%, 17.7%, 37.5%, and 52.2% were prepared. Aluminum pans filled with these solutions were placed on the lower clamp of the Nanobionix whilst a glass probe of
diameter 30 µm, placed firmly on the top grip was lowered onto the solution at a rate of 0.1 mm/sec till the force registered during penetration reached 10 µN after which the probe was allowed to relax for 60 seconds and then pulled back at controlled rates.

6.4 Results

Difference in the structure and the humidity-responses: Viscid silk glue and gumfoot silk glue differ in structures and properties. Viscid silk glue droplets are heterogeneous with a dense polymeric core surrounded by a sparse, translucent mixture of glycoproteins and an aqueous solution of salts (Figure 6.1a). In contrast, gumfoot silk glue droplets appear largely homogeneous with no visible core (Figure 6.1b). These glues also respond very differently to humidity. Viscid silk glue droplets swell by close to an order of magnitude compared to their desiccated volumes (Figure 6.2), while gumfoot silk glue droplets instead coalesce together to form bigger droplets such that the total increase in volume is much less than in viscid silk glue (Figures 6.1c, 6.1d & 6.2). The ‘flow’ and coalescing of gumfoot silk droplets is probably due to the absence of a dense central core, which is hypothesized to act as an anchor for the viscid silk glue droplets thereby keeping them firmly attached to the axial silk fibers [84]. The absence of the core likely explains why glue droplets can be easily removed from the gumfoot silk by adhering onto an adhesive surface, unlike viscid silk glue which is firmly attached to the axial silk fibers and hence returns to the viscid silk after exhibiting the ‘suspension
bridge’ mechanism [84]. The glycoproteins in viscid silk glue behave like a crosslinked network and exhibit viscoelastic solid-like behavior [93]. While, gumfoot silk glue contains water-soluble adhesive peptides [113] and GABamide [79]; the presence or absence of high-molecular weight branched adhesive polymers (proteins) is not known yet.
Figure 6.1| Gumfoot silk glue vs. viscid silk glue (a) and (b) show individual viscid silk thread and gumfoot silk thread spun by Lariniodes cornutus and Latrodectus hesperus, respectively. Capture threads were laid on clean cover slips for both the cases. The difference in the wetting kinetics of the coating peptides and the high-molecular-weight adhesive polymers (probably glycoproteins) gives the appearance of a ‘diffuse core’ in
the gumfoot silk glue droplets. The glue droplets homogenize with time which disappears the core. Also, this core is not observed in pictures of suspended gumfoot silk threads. Scale bar is 20 µm for both the cases. (c) and (d) show a gumfoot silk thread at 0% R.H. and 90% R.H., respectively. It was observed that when a gumfoot silk thread is humidified, the glue droplets flow and coalesce to form bigger droplets.

Figure 6.2] Water uptake of the glues. Change in volume of the viscid silk glue (squares) and gumfoot silk glue (circles) as the silk threads are exposed to a high-humidity environment. Insets a (c) and b (d) show gumfoot silk glue (viscid silk glue) at 0% R.H. and 100% R.H., respectively. Similar to figures 6.1c and 6.1d, inset b shows fewer but bigger glue drops than inset a. Scale bar is 100 µm for all the figures. The uptake of water in viscid silk glue drops is due to the presence of low molecular weight hygroscopic compounds present in the glue. It was experimentally determined that there is no hysteresis in water uptake with humidity cycling (data not shown). In the case of the gumfoot silk glue, however, the order of changing humidity plays a role. While going up in humidity for the first time, the glue drops on gumfoot silk coalesce to form bigger drops and a slight change in total glue volume is observed (circles). Reducing the humidity subsequently restores the original glue volume but obviously not the original number of glue drops. Subsequent humidity cycles are completely reversible in terms of both glue volume and number of glue drops.

Dependence of adhesion on humidity: Capture-thread glue drops swell (to different extents depending on which glue) when exposed to high humidity (Figure 6.2).
This absorbed water dilutes the glue drops, thus improving their wettability. The effect of humidity on adhesion of these glues is investigated by equilibrating threads at different levels of humidity before performing the individual glue droplet measurements. Capture thread, equilibrated at the desired humidity, is immobilized on a glass substrate and a conical glass probe of 10 µm base diameter is brought into contact with its glue droplets. The whole assembly is observed through an optical microscope and is enclosed in a humidity-controlled chamber. The probe is then retracted at constant speeds while the force is recorded as a function of distance (Figure 6.3). To account for the change in modulus (softness) with humidity and to objectively compare the two glues, the normal pre-force for bringing the probe in contact with an individual drop was kept constant for every value of humidity for both glues. After reaching the critical ‘pull-off’ force, the tip releases contact. The critical pull-off forces depend on the rate at which the droplets are stretched. The force-displacement behavior for individual drops during their stretching is shown in Figure 6.3.

The effect of humidity on the adhesive behavior of these glue droplets can be understood by comparing the load-displacement behavior at the same stretching rate (50µm/s) at different values of R.H. (Figures 6.4a and b). For the viscid silk, the glue drops become softer with increasing R.H. (the initial elastic modulus decreases with increase in R.H.). Because the adhesion between the probe and the glue droplets is used to stretch the droplet, the extension of the glue droplets at break is also dependent on humidity. In contrast, humidity does not have any significant effect on the adhesion of...
Figure 6.3 | Effect of humidity on the stretching behavior of the glues. Force-displacement behavior when glue drops of viscid silk (gumfoot silk), equilibrated at 15% R.H. a(b), 40% R.H. c (d), and 90% R.H. e (f), were stretched at 1 µm/s (inverted triangles), 10 µm/s (upright triangles), 50 µm/s (squares), and 100 µm/s (circles).

gumfoot silk glue (Figure 6.4b). The pull-off forces for gumfoot silk glue depend only on the rate of stretching and are independent of the surrounding humidity. Also, despite the larger sizes of the gumfoot silk glue droplets, their extension-to-break values are much lower than that of the viscid silk glue droplets. This might be due to gumfoot silk
glue’s reduced uptake of water compared to viscid silk glue. When the glue ‘pull-off’ forces for
Figure 6.4 | Comparison between viscid silk glue and gumfoot silk glue. (a) and (b) Force-displacement behavior when individual glue drops of viscid silk and gumfoot silk, equilibrated at 15% R.H. (circles), 40% R.H. (squares), and 90% R.H. (upright triangles), are stretched at 50 µm/s, respectively (data acquired from Figure 2). (c) Comparison of the pull-off force obtained from Figure 4a and b with the capillary forces exerted by unentangled PDMS (γ ~ 20 mN/m) and an aqueous solution of composition similar to the viscous coat used by modern orb-weaving spiders to coat their capture threads (γ ~ 40 mN/m). VSS glue denotes viscid spiral silk glue whereas GFS glue denotes gumfoot silk glue. VSS glue, depending on the relative humidity, is represented by box and whiskers outlined by red (15%R.H.), blue(40%R.H.), and green (90%R.H.), whereas, for GFS glue, boxes and whiskers are outlined with black and boxes are filled with the color. PDMS is represented by box filled with purple whereas aqueous solution is represented by box and whiskers outlined with purple. d) Comparison of energy of adhesion between viscid silk glue, gumfoot silk glue, and the $U_{\text{glue}}$ values obtained using the energy model as explained in the supplementary information. Values are obtained by multiplying the area under the force-displacement curve obtained from individual glue drop stretching measurements by 42 (number of glue drops in contact with a 2 mm glass substrate used for the peeling experiments). Even though gumfoot silk does not have 42 droplets per 2 mm length, and thread peeling measurements were not performed with it, values plotted are obtained by multiplying the area under the force-displacement curve by 42, in order to compare it with viscid silk glue and the $U_{\text{glue}}$ values obtained using the energy model. Values are plotted as box and whiskers from 5 measurements each. VSS glue is represented by box and whiskers outlined with black and filled with red (1µm/s), blue (10µm/s), green (50 µm/s), and purple (100 µm/s). GFS glue, depending on the rate of stretching, is outlined by one of the above colors. $U_{\text{glue}}$ values are represented by brown-filled boxes outlined with black.

Both silks were compared with the capillary effect-induced forces of two model viscous liquids (unentangled PDMS, γ = 20mN/m, and a solution similar to the aqueous coating of the viscid silk glue, γ = 40mN/m, respectively), both silk glue forces were two orders of magnitude larger than either liquid, even at 90% R.H stretched at 1 µm/sec (Figure 6.4c). Furthermore, the glue pull-off forces depend on the rate of stretching while the liquid viscous forces are rate-independent. This implies that gumfoot silk glue exhibits viscoelasticity, which, like viscid silk glue, indicates the presence of physical or chemical crosslinks likely caused by high-molecular-weight adhesive polymers. Also, rate-
dependent pull-off forces demonstrate that the viscid silk glue does not lose its viscoelastic character even when diluted and swollen by up to an order of magnitude (i.e. at 90% R.H.).

The area under the load-displacement curve represents the energy required to separate the tip from the glue (referred to as adhesive energy). Figure 6.4d compares the adhesive energies as a function of rate and humidity for both glues. The adhesive energy is higher for faster stretching rates due to viscous dissipative forces. Interestingly, gumfoot silk glue does not adhere as strongly as the viscid silk glue at any humidity. For gumfoot silk, the adhesive energy remains unaffected by the level of R.H., just like its adhesive forces. For viscid silk glue, at the same stretching rates, both adhesive energy and adhesive forces are maximized at intermediate levels of humidity.

Figure 6.5| Peeling of capture silk threads is humidity-dependent. (a) Schematic of the set-up used for thread peeling measurements. (b) Peeling forces when capture threads,
equilibrated at different values of R.H., were separated at 2 mm/s from a 2 mm wide clean glass substrate. Values are plotted as box and whiskers from 10 measurements each. (c) Tensile characteristics of capture threads equilibrated at 0% R.H. (circles), 15% R.H. (squares), 40% R.H. (upright triangles), 65% R.H. (inverted triangles), and 90% R.H. (diamond). Stretching rates in each case were similar to the stretching rates of capture threads during peeling measurements. Values are plotted as mean ± s.d. from 5 measurements each. (d) Energy consumed by all the glue droplets (in contact with a 2 mm glass substrate) in stretching during thread peeling measurements at different values of R.H. Values are determined using the energy model as has been described in the text and are plotted as box and whiskers.

Effect of humidity on the adhesion of viscid silk threads: Humidity dependence of the adhesive energy of these droplets has immense consequences on the functioning of the capture threads. A single capture thread is covered by many of these droplets and peeling of this thread depends on the glue drops and the humidity-dependent mechanical properties of the viscoelastic axial silk fibers. Figures 6.5a and b show the setup used and the force required to peel a single capture thread from a glass surface at a constant rate, respectively. The humidity-dependence of this force at pull-off can be clearly seen. Interestingly, similar to the single drop measurements, the thread peeling forces are also highest at intermediate values of R.H.

The peeling forces depend on the glue drops and the tensile properties of axial silk fibers, and since the tensile properties also depend on the humidity, we need to separate the contributions of each component before we compare the contributions of glue droplets on the adhesion of the silk thread caused by humidity. We have used the energy model developed recently to separate these two contributions. This energy model is based on the principle of conservation of energy and states that the work performed to separate a silk thread from a surface is consumed in stretching the axial silk and in the energy
required to peel the glue droplets off of the surface. The total work on the system \( W_T \) is calculated by integrating the product of the force \( f(h) \) times the infinitesimal height change \( dh \) from \( h \) to \( h + dh \).

\[
W_T = \int_{h=0}^{h=\text{at pull-off}} f(h)dh
\]

The strain energy stored in the thread when it is pulled from its initial position until it separates from the surface, \( U_{strain} \), is given by the following equation.

\[
U_{strain} = (\int_{\varepsilon=0}^{\varepsilon \text{at pull-off}} \sigma(\varepsilon) d\varepsilon) \times \pi \times r^2 \times (L-D)
\]

\( \sigma(\varepsilon) \) is the value of stress at strain, \( \varepsilon \) and \( r \) is the radius of the thread (Figure S1 a). Subtracting \textbf{Equation 3} from \textbf{Equation 2} gives the energy required to separate the glue drops from the surface, \( U_{glue} \).

The tensile properties of the axial silk fibers were measured at different R.H. for calculating \( U_{strain} \) (Figure S1 c). As can be seen, water plasticizes the axial silk fibers making them softer and more extensible. \( U_{glue} \) values were next measured and were compared at different R.H. (Figure S1 d). These values are maximized at intermediate values of R.H. such as 40% R.H. and 65% R.H., as was also observed for single glue
drop adhesive energies. These $U_{\text{glue}}$ values can be compared to the adhesive energies of single glue droplets in order to find an agreement between theoretical calculations and experimental measurements. The energy consumed in stretching one droplet can be equated to the area under the load-displacement curve. Assuming a distance of 40 µm between two large droplets on the capture thread of Larinioides cornutus, there are a total of 42 droplets in contact with the surface. Depending on the humidity and hence the rate at which a droplet gets stretched during a thread peeling experiment, multiplying the work done by one drop at that stretching rate by 42 gives us a value close to $U_{\text{glue}}$ (Figures 6.4d, and 6.5d). The $U_{\text{glue}}$ values lie well in the range of the adhesive energies calculated using the single drop measurements.

Surprisingly, whole viscid silk thread adhesion (Figure 6.5a), just like single droplet viscid silk glue adhesion, maximizes at intermediate levels of humidity too (Figure 6.5b). This pattern is somewhat counter-intuitive since viscid silk threads demonstrate monotonic increase in softness and extensibility with increasing relative humidity (Figure 6.5c), which should promote adhesion. Since the gumfoot silk thread releases its glue upon coming into contact with a surface, whole-thread adhesion measurements were not performed with it.

Physical or Chemical Crosslinking: Viscid silk glue drops act like a viscoelastic solid, which helps the spider in retaining trapped prey long enough to be subdued [93]. The viscoelastic solid nature of these glue drops could be due to either physical or chemical crosslinking. If it is physical crosslinking, like hydrogen bonding or electrostatic interactions, then the glue drops should behave as a liquid at long times at
high R.H.. If it is chemical crosslinking on the other hand, the glue drops should behave as a solid at long times, irrespective of the humidity. To test these predictions, load-

Figure 6.6] Effect of humidity on crosslinkers. Load-relaxation behavior of individual glue drops of viscid silk (gumfoot silk) equilibrated at 15% R.H. a (b), 40% R.H. c (d), and 90% R.H. e (f) stretched by a constant length at rates of 1 µm/s (inverted triangles), 10 µm/s (upright triangles), 50 µm/s (squares) and 100 µm/s (circles). Values are plotted as mean ± s.d. from 5 measurements each. When viscid silk glue is stretched at 100 µm/s at 15% R.H., it releases contact with the tip before stretching 100 µm (Figure 3a), hence load relaxation measurements could not be performed at these conditions. (Figure 6.6a).
relaxation measurements were performed in which the glue droplets, equilibrated at desired humidity, were stretched by a constant distance and the load was allowed to relax (Figure 6.6). For the viscid silk glue the magnitude of the load plateau decreases as the humidity increases. This implies a reduction in the crosslinking density and hence, the elasticity, of viscid silk glue droplets (Figures 6.6a, c, e, and 6.7a). Although this suggests the presence of physical crosslinking, chemical crosslinking cannot be completely ruled out because the relative magnitude of the load plateau at 90% R.H. versus the capillary pull-off forces (measured above) is not known due to limited resolution of the force measurements. The swelling of the viscid glue droplets at high humidity while maintaining their shapes (Figure 6.2), as well as the presence of amino acid sequences similar to elastin and flagelliform spider silk in one of the glycoproteins in the silk produced by Nephila clavipes [52], suggest chemical crosslinking as well.

![Figure 6.7](image)

**Figure 6.7** Effect on glue elasticity. Plateau values, indicative of the amount of elasticity in the glue, reduce with increasing humidity in the case of viscid silk glue (a) but remain constant for gumfoot silk glue (b). Plateau values for gumfoot silk glue are plotted using a fitting function since the values were lower than the resolution of the equipment (1μN).

Gumfoot silk glue droplets, on the other hand, behave like a viscoelastic liquid at all levels of humidity (Figures 6.6b, d, f and 6.7b). Any load plateau is lower than the
resolution of the equipment, which suggests the presence of very little, if any, crosslinking. The easy separation of the glue droplets from gumfoot silk to any substrate to which they adhere contrasts with the formation of a ‘suspension bridge’ and eventual release of the viscid silk glue droplets. In addition, gumfoot silk glue ‘flows’ and coalesces at long times such that the drops lose their shape, as opposed to the viscid silk glue droplets which stay intact. All of these observations support the viscoelastic liquid nature of the gumfoot silk glue.

6.5 Discussion

Both orb web and cobweb spiders depend upon liquid glue droplets for their silk to adhere to insect prey. Both types of spiders use the same sets of glands to produce the adhesive. Aggregate glands evolved initially in orb spiders to coat their elastic capture spirals and then were co-opted during the evolutionary origin of cobwebs to coat the base of gumfoot capture threads. Despite close evolutionary homology, the two bio-adhesives are remarkably different, especially in how they interact with water. For the viscid silk glue in orb webs, the change in the adhesion energy of the glue droplets as a function of humidity is controlled by several competing processes. The hygroscopic salt plays an intrinsic role not only in sequestering water but also in solvating the glycoproteins (Chapter 5). The increase in water content increases the spreading of the glue droplet. This spreading of the glue enlarges its contact area with the surface. In addition, the long-time plateau in the force relaxation measurements also decreases with increase in
humidity. This indicates that the effective crosslink density also decreases with increase in water content. The complexity of the problem is further evident if we consider that the glycoprotein is negatively charged such that changes in concentration of water also change the electrostatic forces and thus the adsorption of the glycoproteins on the glass substrate.

Figure 6.8 | Polymer model to understand the humidity effect. (a) Pull-off energy plotted as a function of concentration of the PEO/water solution at a pull-off rate of 1mm/sec. (b) Energy calculated as area under the load-displacement curve during pull-off plotted as a function of the pull-off rate for concentrations of 13.7% (circle), 17.7% (upright triangles), 35.5% (squares), and 52.2 % (inverted triangles) of the PEO/Water solutions.(c) A schematic of the state of the glue drops at different values of R.H. Chemical crosslinking (red) remains unaffected with changes in humidity while the viscosity and elasticity reduce with increasing humidity. Lubricating action becomes predominant at higher values of humidity.

To simplify the problem and to understand the underlying mechanism, we have designed a polymer model consisting of high molecular weight polyethylene oxide (PEO)
dissolved in water. Measurements, similar to those conducted on individual glue droplets were performed on PEO/water solutions of different concentrations. For the same pull-off rates, the energy of adhesion (i.e. area under the load-displacement curve) increased with higher water concentration, reached a maximum, and then reduced with further increase in humidity (Figure 6.8a,b).

This trend captures the results obtained for the viscid silk glue drops produced by spiders. The optimum concentration of water for adhesion can be explained by two competitive mechanisms. The total work done in pulling the probe out of the PEO solutions is similar to the empirical equations used to describe the rate-dependent work done in peeling viscoelastic adhesives $\sim G_0 (1 + f(R,T))$ [19]. $G_0$ is related to the thermodynamic work of adhesion and $f(R,T)$ is a term that reflects the energy expended in irreversible processes that include elastic and viscous forces. At lower concentrations of water, the viscosity and elasticity are very high, which tends to increase the contribution of the irreversible work of adhesion. However, the spreading rates are very slow at these lower concentrations thereby reducing the area of contact. At high concentrations of water, the spreading rates are fast, but the viscous and elastic forces are lower. In addition, the effectiveness of the interfacial contact of the glycoproteins is reduced at high water concentration due to lubrication. The interplay between these two competing effects leads to an optimum stickiness at intermediate humidity. Figure 6.8c shows a schematic of the state of the glue drop at different values of humidity. The chemical crosslinking (red squares) remains unaffected whilst the water content of the drop increases at higher values of R.H., which reduces the glue drop’s viscosity and elasticity and also lubricates its interface with the glass substrate. At intermediate
humidity (40% - 60% R.H.), these parameters are optimized such that the adhesion is maximized. Considering that the salts are the predominant hygroscopic component of viscid silk glue, the optimal R.H. that maximizes adhesion should largely depend on the concentration of salts in the viscous coat. Shifts in salt concentration would therefore provide an easy mechanism for evolution to act on the adhesion of spider silk glue, particularly across species whose habitats vary in ambient humidity.

Significantly less is understood about the chemical composition of the gumfoot silk. The weak effect of humidity on the droplet size, the ability of the droplets to flow, coalesce, and separate easily from the gumfoot thread, and the display of a viscoelastic liquid-like behavior unaffected by humidity, are all behaviors in stark contrast to the viscid silk glue. We hypothesize that the polymers (probably glycoproteins) in the glue are not crosslinked, which results in the absence of the central dense core seen in viscid drops, easy separation of the glue from the gumfoot silk, and the flow and coalescing of these glue droplets. Also, while viscid silk glue maintains ‘fluidity’ due to the water absorbed by the hygroscopic salts (Chapter 5), gumfoot silk glue instead maintains fluidity due to the presence of the low-molecular-weight water-soluble coating peptides (Spider Coating Peptides [113]). These differences explain why gumfoot silk glue’s adhesion and elasticity are resistant to changes in humidity. Water swells the glue slightly, which causes enough increase in fluidity to make these droplets flow, but not significantly enough to cause a change in their structure or adhesion. This humidity-resistant strategy works very well for these spiders since widow spiders must maintain adhesiveness in their glue across a wide range of environments, some of which are quite arid. The inability of viscid silk glue to adhere at low humidity is the reason why the
individual glue drop measurements with viscid silk were performed at 15% R.H. and not 0% R.H. At 0% R.H, the stiffness of the viscid silk glue did not allow the microscopic glass probe to penetrate inside it at the pre-force range used for these measurements. **Figure 6.5d** shows the difference in glue adhesion at 0% R.H. and 15% R.H.).

A second possible adaptive explanation for the evolutionary shift in humidity responsiveness of spider glues during the origin of cobwebs relates not to microhabitats but instead to the structures of the webs themselves – humidity resistance could prevent ‘local’ supercontraction in the cobweb. The entire capture spiral of an orb web is encased in its highly hydroscopic glue and water therefore infiltrates the flagelliform silk core, causing it to supercontract. This is an essential feature that helps to make the silk soft, highly extensible and resilient. In contrast, cobweb silk glue is laid upon only a small portion of the gumfoot capture thread, which is composed of dry major ampullate silk threads. This silk can shrink as much as 50% of its length when wetted and generate stresses in excess of 100 MPa [59]. If whole webs supercontract then the stresses generated in individual threads can be equalized, thereby maintaining the structure and function of the web (Boutry and Blackledge, unpublished). However, this would not be the case if gumfoot glue drops were hydroscopic and highly responsive to humidity because they coat only the bottom portion of a gumfoot thread. If just this region supercontracted then it could cause the separation of the gumfoot thread from the surface because the stress exceeds the strength of the piriform disk attaching it to the substrate (results not shown). Local supercontraction of individual threads would also alter the tensions of threads in the web and likely attenuate the vibration-transmission efficiency due to the softening of the gumfoot thread.
Nature exhibits many intriguing strategies that take advantage of water, the most common liquid on earth. Gecko toes when exposed to high humidity adhere better to surfaces [42]. Tree swallows exhibit brighter colors when wet [108]. Spiders have used a combination of synergistic materials to promote or maintain high adhesion to capture prey. Here, we have shown that cobweb-weavers, using a cocktail of short peptides and long adhesive polymers (likely glycoproteins), maintain the adhesion of their prey capture glue over a large variation in humidity. The combination of the humidity-resistant glue and strength of the dry major ampullate silk fiber is necessary for catching pedestrian insects. On the other hand, the glue produced by orb-weavers is highly responsive to water. Hygroscopic salts present in the viscid glue of orb spiders make it highly humidity-sensitive. Humidity swells these glue droplets and promotes the spreading of viscoelastic glycoproteins present therein to increase the adhesive contact with the substrate. Viscid silk threads take advantage of the synergistic combination of this glue and the underlying flagelliform silk fibers in catching preys flying into the web at high velocity. The understanding of how nature takes advantage of these strategies to enhance the survival and proliferation of their species provides a plethora of ideas for designing synthetic adhesives that work in presence of water or humidity.
CHAPTER VII

SPIDER SILK INSPIRED FUNCTIONAL MICROTHREADS

7.1 Abstract

We employ the adhesive web building strategy used by modern orb-weaving spiders to produce functional micro-threads that are similar in structure (beads-on-a-string morphology) and adhesive properties to the capture-silk threads of the spider web. The diameter and spacing of droplets (beads) are controlled by varying the viscosity, velocity, and surface tension of the coating fluid. Using these functional threads, we also describe the behavior of the beads-on-a-string morphology (BOAS) during contact (mimicking the collision of an insect with the web) and during separation (mimicking insect rescue from the web). Our results show that the BOAS structure performs better than a cylindrical structure for adhesion, which may explain why this morphology is so prevalent in spider webs despite the cost of increasing the visibility of the web [115].
7.2 Introduction

Spiders display innovative behavioral strategies in conjunction with micron-size custom-made adhesive silk threads incorporated into prey capture webs [45]. The proteins as well as low-molecular-mass organic and inorganic materials used in these webs evolved over millions of years into a class of natural adhesives with outstanding properties. Multiple types of adhesives are often used in a single spider web; however, the adhesives used to capture prey have received the most attention. Silk threads spun by modern orb-weaving spiders to capture prey consist of a beads-on-a-string (BOAS)[62]-like morphology, where the beads of glue are composed of adhesive polymeric glycoproteins [80, 93, 94] and low-molecular-weight hygroscopic compounds [78]. The string is comprised of a pair of soft and highly extensible viscoelastic axial silk fibers [100]. The BOAS threads are produced from triads of spigots that lie on the posterior spinnerets of spiders. Each triad is composed of a gland that produces an axial silk fiber (flagelliform gland), two glands which secrete glue (aggregate gland) and their respective spigots. The spigot from the fiber gland is arranged between the spigots of the glue glands such that the glue and fibers are simultaneously extruded [49] and glue coats the fiber. The composition of the glue, its physical characteristics and the spinning conditions of the silk produce an initially cylindrical coating of the glue on the axial silk which breaks down into an equally-spaced micron-sized array of glue droplets due to Rayleigh
instability. After a period of time, the glue droplets become more viscous and develop elasticity, which become important in enhancing adhesion [93].

Interestingly, even though Rayleigh instability results in reducing the surface area of the adhesive coating, the BOAS structure is prevalent. In addition, the BOAS thread is more visible than the cylindrical thread - an undesirable characteristic in a trap. The success of BOAS morphology is also evident through other examples, especially by its presence in the gumfoot silk threads [114] spun by the cobweb-weaving spiders, the evolutionary descendents of modern orb-weaving spiders.

Here, we employ the strategy utilized by modern orb-weaving spiders to produce functional micro-threads (hereafter referred to as 'functional-threads'). We also tune the magnitude of functionality imparted by varying the velocity, viscosity, and surface tension of the coating material. In general, higher viscosity and velocity and lower surface tension of the fluid result in the formation of bigger and farther-spaced droplets. Using these functional-threads, we describe the behavior of the BOAS and cylindrical morphologies during contact (mimicking collision of an insect) and during separation (mimicking insect rescue) and show that the BOAS structure is better suited than a cylindrical structure for adhesion, despite higher visibility of the adhesive thread in the web.

7.2 Methods

Preparation of functional-threads: Nylon fibers (gifted by Goodyear, Akron, OH) of diameter 30 µm were vertically withdrawn, at a controlled velocity, from a reservoir
filled with poly (dimethylsiloxane) (PDMS) (equal parts of Sylgard 528A and B provided by Dow Corning). The cylindrical coating of PDMS spontaneously breaks into an array of droplets. The thread is placed in a vacuum oven at 80 °C for 2 hours to cure the PDMS. To show the effect of viscosity on drop dimensions and spacing, uncrosslinked PDMS of kinematic viscosities 10 cst, 100 cst, and 1000 cst (10^{-5} \text{ m}^2/\text{sec}, 10^{-4} \text{ m}^2/\text{sec}, and 10^{-3} \text{ m}^2/\text{sec}, respectively) were used (Dow Corning).

Measurement of adhesion force: 16 mm samples of the cured threads, mounted across the gap of a U-shaped cardboard piece, were clamped onto the top grip of NanoBionix® (Agilent Tech., formerly MTS) as shown in Figure S1. A 2 mm-wide clean glass plate was clamped onto the bottom grip. The thread is pushed onto the glass plate to a force of 20 µN at a rate of 0.1 mm s^{-1} (displacement-controlled loading), held there for 60 seconds, and then pulled away from the substrate at a fixed rate (2mm/sec for Figures 7.2c and 7.2d, and 0.5mm/sec and 2mm/sec for Figure 7.4 inset), while the force-displacement response is recorded (Figure 7.3). Force is measured by the displacement of the actuating transducer in the NanoBionix. The maximum displacement of the transducer is ± 1mm. The force registered just before the thread releases contact with the glass plate is recorded as the adhesion force. 10 samples were tested three times each. Values are plotted as mean ± S.D. from 30 measurements each.

Measurement of the tensile properties: Independent tensile tests on the threads were performed, by clamping a thread onto the top and bottom grips of the NanoBionix, to find out the strain energy stored (area under the stress-strain curve) when the thread is stretched (Figure 7.3). The maximum strain to determine the energy stored in the thread
was determined from the maximum strain experienced by the thread during the adhesion measurements. These tensile tests were performed at the same stretching rates that the threads experienced during adhesion measurements, to account for any viscoelastic effects. Strain energy values were determined from testing ten 16-mm long thread samples. The equation used to calculate the contribution of the energy stored in stretching the thread is discussed in the main text.

7.3 Results and discussion

A continuous micro-fiber (Nylon) is pulled vertically out of a reservoir containing PDMS at different velocities. We choose PDMS as a liquid because it has low surface tension and the effect of gravity on the droplets is negligible (Bond number \(<< 1\)). Depending on the capillary number, \(Ca\), the cylindrical coating breaks down into an array of droplets due to Rayleigh instability. The coated fiber is then cured by heating at 80° C to stabilize and immobilize the droplets. Figures 7.1a and 7.1b show a capture thread spun by \textit{Argiope trifasciata} and functional-threads formed by coating nylon fiber with PDMS.
Figure 7.1] Fabrication and tuning of ‘functional-threads’. a) and b) show a capture spiral thread spun by Argiope trifasciata and a thread produced by withdrawing a nylon thread out of a reservoir filled with PDMS, respectively. c) Shows the effect of velocity of coating on drop dimensions when nylon threads are coated with PDMS of kinematic viscosity 1000 cst at velocities of 690 µm-s^{-1}, 2460 µm-s^{-1}, and 9460 µm-s^{-1} (left to right). d) Shows the effect of PDMS viscosity on drop dimensions. Nylon threads are coated, at 9460 µm-s^{-1}, with PDMS of kinematic viscosities 10 cst, 100 cst, and 1000 cst (left to right). Capillary number increases from left to right. Scale bars in c and d are 150µm and 50µm, respectively.

The adhesion of the fibers can be easily tuned by varying the capillary number. Upon withdrawing a fiber from the reservoir, the thickness of the entrained cylindrical film, \( e \), for Bond number \( \ll 1 \) (which is the case here), is given by the following equations [116]

\[
e = \begin{cases} 
1.34dCa^{2/3}, & Ca \ll 1 \\
\frac{1.34dCa^{2/3}}{1-1.34Ca^{2/3}}, & Ca \sim 1
\end{cases}
\]  

(1)

Here, \( Ca = \eta V/\gamma \) and \( d, \eta, \gamma, \) and \( V \) are the radius (of the uncoated fiber), viscosity, surface tension, and velocity of the coating, respectively. Plateau [117] and Rayleigh
[118] showed that the initially cylindrical coating breaks into an array of drops such that the wavelength of the array, $\lambda$, as well as the radius of the sphere, $R$, are both dependent on the thickness of the cylindrical coating $e$.

$$\lambda > 2\pi (d+e)$$  \hspace{1cm} (2)

$$R = \left\{ \frac{3\lambda}{4} ((d + e)^2 - d^2) \right\}^{1/3}$$  \hspace{1cm} (3)

For the sake of simplicity in expressing a relation between the thickness of the cylinder and the dimension of the drop formed, the assumptions made here are that the drops are spherical and that there is no fluid ‘bridge’ between two drops. In reality, however, the drops formed are paraboloidal (if the fluid wets the fiber), and a cylindrical film of thickness $<<$ thickness of the initial cylinder bridges between two drops.

In essence, by varying $\eta$, $\gamma$, or $V$, one can change $Ca$. Changing $Ca$ will change the thickness of the coating and hence the radius of the cylinder, which will determine the wavelength $\lambda$ of the array of spheres of radius $R$. Using this principle, the size and spacing of the drops on the functional-threads were tuned by varying the capillary number. Figure 7.1c shows the effect of velocity, $V$, on the size and the spacing of the drops. The volume of the drop as well as the spacing between the droplets increases with increasing velocity of the coating. Figure 7.1d shows the effect of viscosity, $\eta$, of the coating on the volume and the wavelength of the droplets. Similar to the velocity, increasing the viscosity also results in higher drop volume and longer wavelength.
Figure 7.2] Testing of functional threads. a) Shows the suspension bridge formed when a capture silk thread spun by Larinioides cornutus is separated from a glass surface. Interestingly, the functional-threads produced by coating Nylon threads with PDMS behave similarly and also exhibit the formation of a suspension bridge-like structure when separated from a substrate (shown in Figure 7.2b). c) Shows the adhesion force of a functional thread (PDMS-coated nylon thread) as a function of the capillary number (Rate of separation of thread from the substrate = 2 mm-s\(^{-1}\)). d) Shows the effect of capillary number on the adhesion energy of the PDMS drops. The energies were determined using a recently developed energy model\(^5\). The error bars are calculated from the error in \(W_T\) and the \(U_{\text{Strain}}\) from 30 measurements each.

The modern orb-weavers have developed an intriguing structure for their capture threads, utilizing glue droplets as adhesive beads on a very elastic silk thread. The glue droplets behave like viscoelastic solids [93] and can withstand large extension as shown in Figure 7.2a. The ‘suspension bridge’-like structure [90] increases the peeling force and makes the capture threads very sticky. This was one of the reasons we chose to use PDMS as the fluid to mimic this morphology, as cross-linked PDMS is elastic and stretchy. Figure 7.2b shows an optical image of the functional-thread as it is pulled-off
Similar to the capture silk threads produced by spiders, a suspension bridge-like structure is observed in the functional-thread (Figure 7.2b). The adhesion forces required to peel the functional-threads are shown in Figure 7.2c. Higher capillary number was accompanied by higher force of adhesion. Interestingly, capture silk threads spun by different orb-weaving spiders also show similar behavior – generally, capture threads with bigger and farther-spaced drops demonstrate higher adhesion [90].

Figure 7.3| Schematic of the Set-up. Figure shows a schematic of the set-up used for adhesion. The dotted line bridging the gap between the cardboard legs shows the initial position and unstrained length \( l = 16 \text{ mm} \) of the capture/functional thread. The width of the clean glass plate fixed onto the lower clamp is 2 mm. The thread is pushed onto the glass plate to a force of 20 µN at a rate of 0.1 mm s\(^{-1}\) (displacement-controlled loading), held there for 60 seconds, and then pulled away from the substrate at a fixed rate (2mm/sec for Figures 2c and d, and 0.5mm/sec and 2mm/sec for Figure 3 inset), while the force-displacement response \( (f(h)) \) is recorded every 0.001 seconds. Force is measured by the displacement of the actuating transducer. Just before the thread releases contact with the substrate, the last few drops (green) in contact with the glass plate stretch and give the appearance of a suspension bridge, as shown in the schematic (stretching of glue drops is exaggerated, compared to \( h_f \) for the sake of visibility). The force registered
just before these last drops release contact with the class plate \( f(h) \) is recorded as the pull-off force (adhesion force shown in figure 7.2c). Figures 7.2a and 7.2b also show suspension bridges formed by spider capture silk and functional threads, respectively.

It has been shown that the force of adhesion depends on the mechanical properties of both the fiber and the glue droplets \([93]\). The contributions from the fiber and the glue can be separated by using a recently developed energy model. The work performed to pull a thread off a surface is consumed in stretching of the axial fiber and the energy required to peel the droplets from the surface. Figure 7.3 shows a sketch defining the variables used in the energy model. The total work on the system \( W_T \) is calculated by integrating the product of the force \( f(h) \) times the infinitesimal height change \( dh \) from \( h \) to \( h + dh \).

\[
W_T = \int_{h=0}^{h=hf} f(h)dh
\]  

The strain energy stored in the thread when it is pulled from its initial position until it separates from the surface, \( U_{\text{strain}} \), is given by the following equation:

\[
U_{\text{strain}} = \int_{\varepsilon=0}^{\varepsilon=\varepsilon_f} \sigma(\varepsilon)d\varepsilon
\]  

\( \sigma(\varepsilon) \) is the value of load at displacement \( \varepsilon \), as measured from independent tensile tests. Here, the assumption is that the length of thread adhered to the substrate at any time during the adhesion measurement is negligible compared to the total length of the thread, which is reasonable since the width of the substrate (2 mm) is much less than the length
of the thread (16 mm). Subtracting Equation (5) from Equation (4) gives the energy required to separate the glue drops from the surface, $U_{\text{glue}}$. Figure 7.2d shows the energy of adhesion of the glue drops as a function of the capillary number. The increase in the energy of adhesion of the glue drops with increasing capillary number indicates that the increase in force of adhesion is due to the glue drops and not because of the difference in tensile properties of the thread. The extent of functionality (adhesiveness in this case) imparted to a fiber can thus be easily tuned by varying the capillary number.

Figure 7.4 BOAS versus Cylindrical morphology. Shows the contact area established on glass by equal volumes of cylinder and the eventually formed droplets, calculated assuming JKR theory which is applicable here since the nylon fiber is coated with PDMS (Sylgard 528 A and B) which, after crosslinking, becomes elastic. The volumes of the cylinders and spheres were calculated using volume conservation on the dimensions of the glue drops on the capture spiral threads spun by Cyclosa turbinata (●), Leucauge venusta (■), Metepeira labyrhythmia (▲), Araneus pegnia (▼), Argiope trifasciata (◄), and Araneus Marmoreus (►)12. Solid symbols represent spheres whereas the corresponding hollow symbols represent cylinders. Spheres establish higher contact area than cylinders for the same loading force. Inset in Figure 7.4 compares the adhesion of freshly spun capture threads (the coating is still cylindrical) (hashed bars) versus capture threads in which the coating has beads-on-string structure (solid bars), at different rates of
pull-off (speed at which the thread is separated from the substrate). Glue droplets cause the capture threads to adhere many times stronger than the cylindrical glue coating.

Interestingly, when a newly spun capture silk thread (the glue coating is still cylindrical) spun by *Larinioides cornutus* is brought into contact with a clean glass plate and then separated from it, the force of adhesion measured at separation is around three times lower than when the glue coating has broken into an array of droplets (*Figure 7.4, inset*). The difference in adhesive forces can be attributed to higher contact area established by the glue droplets than the glue cylindrical morphology *and* the higher energy dissipated in separating the glue droplets than in separating the glue cylinder, since peeling glue droplets from a surface will have multiple crack-initiation, crack-propagation, and crack-arrest events, whereas peeling a cylinder will require only one of each. Using the functional threads as an example, we studied the effect of these factors (interfacial contact area established *and* energy dissipation during separation) on the adhesion of both morphologies (cylinders and spheres) in an attempt to understand the advantage of the BOAS morphology on the capture silk threads. Interestingly, all of the difference in force of adhesion, in actual spider capture silk, cannot be attributed to the difference in morphology because in capture silk thread, the glue, after being coated onto the silk fibers, undergoes crosslinking to stabilize and immobilize the glue droplets onto the silk fibers. This contrasts the situation when the glue is still cylindrical and the glue is not crosslinked. Nonetheless, morphology of the glue drops plays a significant role in influencing the adhesive capabilities of the capture threads.
Considering a linear elastic model, we can calculate the differences in contact areas between the BOAS morphology and the cylindrical coating of similar volume (mimicking insect capture). For the sake of simplicity, we assume the glue drops to be spherical (in reality, the glue drops produced by spiders are paraboloidal). According to the JKR theory, the contact radius, \( a \), of the circle formed by pressing a deformable sphere onto a rigid flat surface is given by \([119]\)

\[
a^3 = \frac{R}{K} \left( P + 3\pi WR + \sqrt{6\pi WR P + (3\pi WR)^2} \right)
\]  

In the JKR model, \( P \) is the applied load, \( R \) is the radius of curvature of the sphere, \( W \) is the adhesion energy of the sphere with the substrate, and \( K \) is related to the effective elastic modulus (\( E_{\text{eff}}, K = 4E_{\text{eff}}/3 \)). In the case of the cylindrical thread, a contact rectangle of length, \( 2l \), and width, \( 2b \), is formed upon pressing a deformable cylinder on a rigid flat surface and is given by \([120]\)

\[
\frac{3\pi b^{1.5}}{8} = \frac{P}{Klb^{0.5}} + \frac{6\pi W}{\sqrt{K}}
\]  

Here, \( S \) is the radius of the cylinder and other terms are similar to those described in equation 5. Figure 7.4 compares the interfacial area, calculated assuming JKR contact, established by a cylinder and the eventually formed spheres, with a rigid flat substrate. Since the cylinder breaks into an array of spheres, the volume of sphere is equal to the volume of one wavelength of cylinder. For the same loading force, a sphere establishes
higher contact area than a cylinder of equal volume (Figure 7.4). For comparing the contact areas established by spheres and cylinders (Figure 7.4), dimensions were calculated by equating the sphere and cylinder volumes to the volumes of actual glue droplets (which are paraboloidal) produced by different species of modern orb-weaving spiders.12 Table 1 of this work by Opell gives the drop length ($l_d$), distance between two drops ($d_d$), and drop width ($w_d$), for glue drops produced by six species of modern orb-weaving spiders. The glue droplet volume, calculated using the integral of the equation of a parabola, is given in its simplest form by the following equation

\[ \text{Drop Volume} = (2\pi w_d^2 l_d) * 15 \]  \hspace{1cm} (8)

This drop volume was equated to the volume of one wavelength of a cylinder and also to the volume of the sphere, using the philosophy that an initial cylindrical coating breaks up into an array of droplets.

\[ \text{Drop Volume} = (2\pi w_d^2 l_d) * 15 = \pi * S^2 * (l_d + d_d) = 4/3 \pi * R^3 \]  \hspace{1cm} (9)

Using the above relation, S and R were calculated. These values were used in equations 5 and 6 to calculate ‘a’ and ‘b’. The system uses a PDMS/glass interface, so ‘W’ in these equations is taken as 70 mJ/m² and ‘E_{eff}’ as 2.4MPa. In equation 7, w is the width of the sample which has been taken as $w_d$. The wavelength of the sample, s ($\lambda$ in previous equations) is taken as $l_d + d_d$. 

119
The glue coatings produced by orb-weaving spiders, as well as the crosslinked PDMS coating on the functional-threads, exhibit elasticity and the elastic model in these calculations is used to illustrate that the sphere geometry (BOAS threads) results in higher contact area with the substrate.

In addition to the higher surface area established by the array of spheres, the BOAS morphology adopted by spiders also has a higher force required to separate the array of spheres from a surface (mimicking insect rescue). The higher separation force is due to the multiple crack-initiation, crack-propagation, and crack-arrest events as opposed to a single event when a cylinder is separated from a surface. Kendall [121] has showed that different interfaces between materials of different thicknesses have a considerable effect on crack propagation: when a crack meets a thicker material, it experiences transient retardation, whereas when a crack meets a thinner material, it experiences transient acceleration. Hence, periodic structures (like spider capture silk and functional threads) substantially increase static interfacial fracture energy through arresting cracks at a thicker interface. Moreover, the dynamic interfacial fracture energy, i.e. resistance to a moving crack, is also raised due to fluctuations of crack speed at a number of periodically spaced interfaces. Kendall’s analysis on peeling tapes suggests that the spacing and diameter of the glue drops are important in increasing the peeling force. We illustrated the importance of this hypothesis by referring to the works of Ghatak and Chaudhury [122].

For the sake of simplicity, the array of drops can be roughly approximated as a one-dimensional adhesive film having equi-spaced incisions on it (distance between
incisions = s). For separating a smooth surface of a finite flexural rigidity D, the stress
decay length, \( \kappa^{-1} \), is given by [122]

\[
\kappa^{-1} = \left( \frac{2w^3}{12\mu} \right)^{1/6}
\]

Here, \( D = 0.02 \text{ N-m} \), \( \mu = 1 \text{ MPa} \) and \( w \) was taken to equal the width of a single glue
droplet, while \( s \) was taken as the wavelength of the droplets. For the dimension of the
array shown in Figure 7.4, \( s_K \) (a dimensionless parameter defined in, which describes the
thickness and the wavelength of the array) for the array of beads varies from 0.3 – 1.3,
while separating a cylinder from the same surface would have \( s_K = \infty \). It has been shown
that energy required to separate the film reduces as \( s_K \) increases\textsuperscript{[122]}, which implies that
the energy required to separate a BOAS thread will be higher than that required to
separate a cylindrical thread. Higher energy dissipated while separating a BOAS thread,
together with the higher interfacial surface area established by the BOAS morphology
demonstrates that the BOAS structure exhibits higher adhesion than the cylindrical
structure.

7.5 Conclusions

In summary, we have mimicked the strategy used by orb-weavers to develop
functional micro-threads with excellent adhesive properties. By varying the capillary
number, the structure and morphology of the functional-threads can be controlled. The
simplicity and scalability of this method over conventional methods used to produce such structures – template-based synthesis [123], vapor-phase synthesis [124], solution-phase deposition [125], and co-axial electrospinning [126], allows rapid and easy large-scale fabrication of one-dimensional BOAS structures with a wide range of compatible component materials. The BOAS structure establishes higher interfacial contact area than a cylindrical morphology, for the same applied load. Also, the energy required to separate a BOAS thread is higher on account of multiple crack-initiation, crack-propagation, and crack-arrest events. These results demonstrate that the BOAS structure has higher adhesion than a cylindrical morphology, which may explain why the BOAS morphology is seen in multiple species of spider and over evolutionary history.
8.1 Abstract

Spiders’ three-dimensional cobwebs can target both walking and flying prey. While the scaffolding silk and supporting framework can sometimes entangle flying insects, gumfoot silk threads function as spring-loaded traps that pull walking prey off the ground and into the web. The scaffolding silk needs to withstand the impact of the prey and support the whole cobweb, including the gumfoot silk. In contrast, the gumfoot silk needs to easily detach from the substrate at its gluey base when contacted by prey. Here, we show that spiders accomplish these divergent demands in mechanical performance by creating silk attachment discs of two distinct architectures using the same pyriform silk secretions. A ‘staple-pin’ architecture is used to firmly attach the scaffolding silk to the substrate and, a previously unknown, ‘dendritic’ architecture is used to weakly attach the gumfoot silk to the substrate. By modifying the architecture of the pyriform silk
secretions spun by cobweb weavers, the gumfoot attachment discs adhere weakly, triggering a spring-loaded trap, while the scaffolding discs adhere so strongly that the major ampullate scaffolding threads break instead of detaching. We show how Kendall’s tape-peeling model describes the differences in peeling force for these two architectures and how these design principles can be incorporated into synthetic attachments. Discovering how spiders use silk attachment to functionalize their webs therefore reveals important design principles for controlled adhesion.

8.2 Introduction

Unlike the all-in-one fiber spun by the ‘web-shooters’ of Spider-Man that is stiff, strong enough to support his weight, and also sticky enough to adhere to any surface, real spiders attach their major ampullate (MA) silk dragline fibers to surfaces using attachment discs spun from pyriform silk [127]. These attachments allow spiders to move safely from place to place while secured to a dragline, and to attach their webs to a variety of surfaces. Cobweb-weaving spiders spin three-dimensional webs that can target both walking and flying prey [59]. While the scaffolding silk can act as a ‘catching net’ for flying prey, the gumfoot silk threads pull the walking prey off the ground and into the web [59, 60]. This requires that, on being agitated by the momentum of the incoming prey, the scaffolding silk remains firmly attached to the ceiling while the gumfoot silk threads easily detach from the surface. Here, we show that cob-weaving spiders accomplish these contradictory requirements by creating attachment discs of two distinct
architectures using the same pyriform silk. Previously known ‘staple-pin’ architecture is used to firmly attach the scaffolding silk to the ceiling and, a previously unknown, ‘dendritic’ architecture to weakly attach the gumfoot silk to the substrate. Interestingly, orb-weavers (evolutionary ascendants of cob-weavers) also use the ‘staple-pin’ architecture to firmly attach their webs to different substrates. Peeling tests conducted on discs confirm that gumfoot discs adhere much weakly than the scaffolding discs. We explain the difference in adhesion strengths of the two architectures by simulating the two attachment discs and using Kendall’s classical tape-peeling model. These results provide unique ideas and design principles for controlling structure of adhesives in the design of novel tunable adhesives for various biomedical and material science applications.

8.3 Methods

Disc sample preparation: Scaffolding disc samples were produced by making the spider (*Achaearanea tepidariorum, Larinioides cornutus, Latrodectus Hesperus, or Nephila clavipes*) walk on a clean glass slide layered with a single Nylon thread (diameter 30μm) such that the spider spins the scaffolding disc symmetrically on the Nylon fiber (Figure 8.4a). Gumfoot disc samples were prepared by lacing a a cobweb-weaver’s cage with glass slides and using a flag (Figure 8.4b) to collect the gumfoot samples with the gumfoot disc. For acquiring an SEM image (JEOL) of the discs, spiders
were made to walk and spin a disc on an SEM stub, and for the gumfoot disc case, the cages were layered with SEM stubs.

Adhesion testing on discs: Nylon threads used for the scaffolding disc was fixed on the top clamp of NanoBionix (Agilent tech.) while the glass slide with the scaffolding disc was fixed on the bottom clamp. The nylon thread was pulled on perpendicular to the plane of the disc at 2mm/sec while the load-extension behaviour was recorded. For testing the gumfoot disc, gumfoot threads were fixed on the top clamp and the glass slide with the gumfoot disc was fixed on the bottom clamp. The gumfoot thread was pulled perpendicular to the place of the disc at 2mm/sec while the load-extension behaviour was recorded.

Adhesion testing on simulated discs: Strips of a stretchable tape (3M, MMM8884) were used to attach a strand of nylon threads on a clean glass plate in the manner described in the text and shown in Figures 8.6a and 8.6b. For the adhesion testing, the glass plate was fixed on the bottom clamp of an Instron, while the Nylon strand was fixed on the top clamp and was pulled perpendicular to the plane of the tape strips at a rate of 50 mm/min, while the load-extension behaviour was recorded.

8.4 Results

The cob-weaving spider *Achaearanea tepidariorum* spins the same type of attachment disc used to secure scaffolding silk when it is made to walk on a glass slide
and secures its dragline. Multiple pyriform gland spigots surround the major ampullate (MA) gland spigot [128] such that the dragline silk produced by spiders is attached to surfaces using multiple nanofibers that are composed of two components that form a core and shell [129]. For sampling the gumfoot disc, the spider’s cage is layered with glass slides and samples are collected after the cobwebs are spun. Figure 8.1 shows SEM micrographs of the scaffolding disc and the gumfoot disc. The difference in the

Figure 8.1 | Attachment disc morphology matches different functions. Figure shows SEM images of scaffolding disc (a) and gumfoot disc (b) spun by the cobweb-weaving spider Achaearanea tepidariorum. Insets show optical microscope images of the respective discs. The black arrows point at dragline silk (MA silk) in Figure a and its inset, while the white arrows point at pyriform fibers, arranged in a ‘staple-pin’ architecture, attaching the dragline silk to the surface. Black arrows point at gumfoot thread (MA silk covered with aggregate glue) in Figure b and its inset while the white arrows point at pyriform fibers, arranged in a dendritic architecture, attaching the gumfoot thread to the surface. Scale bars in both the figures are 100 µm.

architecture of the discs is clear. The scaffolding disc resembles a ‘staple-pin’ architecture while the gumfoot disc shows a ‘dendritic’ architecture. Each pyriform fiber is coated with a fluid that likely facilitates adhesion with both smooth and rough surfaces
(Figure 8.2). The number of pyriform fibres in staple-pin discs is much higher than in
dendritic discs (1550 ± 100 vs. 188 ± 20), which translates into a greater number of load-
bearing attachment points in staple-pin discs. (There are observable intra-species
differences in the size of the discs and the density of pyriform fibers within each disc. To
avoid these differences, these numbers were calculated from 5 scaffolding discs and 5
gumfoot discs spun by the same spider on the same substrate (clean glass plate). Numbers
are plotted as mean ± S.D. from 5 samples.

Moreover, in gumfoot discs, pyriform fibers are bound to the surface only at the
periphery of the discs and are instead suspended in air as they converge on the gumfoot
threads (Figure 8.3). This results in only the thinnest pyriform fibers in contact with the
surface, since pyriform fibers bifurcate successively, becoming thinner towards

Figure 8.2| Pyriform fibers have two components. Figures 8.2a and 8.2b show the
extremities of a scaffolding disc and a gumfoot disc spun by Achaearanea tepidariorum.
Pyriform fibers are a composite system containing an axial fiber coated with a fluid. The
black arrows point towards the fluid. The white arrows in Figure S1a point towards the
hair-pin bends at the extremity of a scaffolding disc indicating that the spider’s spinnerets
sweep back and forth, across an MA silk fiber, with their pyriform glands when spinning these discs. Scale bar in both these images is 5 µm.

the periphery of the discs (Figure 8.3). In contrast, in scaffolding discs, most of the length of the pyriform fibers, which are thicker than in gumfoot discs, is in contact with the surface.

Attachment discs are formed without the involvement of legs [130] so that differences result solely from movements of the abdomen and spinnerets. The anterior spinnerets are brought into direct contact with the substrate and are rubbed against it while a secretion is exuded through the pyriform gland spigots [130]. The different architectures of the attachment discs therefore imply different movement patterns of the anterior spinnerets against the substrate. Based on high resolution images of the discs, and the hairpin bends as shown in
Figure 8.3| Pyriform fibers suspended in air in a gumfoot disc. Figure shows an SEM image of a gumfoot disc spun by Achaearanea tepidariorum. The white arrows point at the pyriform fibers that are suspended in the air thereby reducing contact with the surface. The black arrows point towards the fibers that are attached to the surface. A gumfoot disc is attached to the surface only at its periphery. Moving towards the periphery, pyriform fibers split successively, such that fibers that are attached to the surface have the finest diameters. The scale bar in this image is 20 µm.

Figure 8.2, we postulate that the spinnerets ‘sweep’ back and forth across an MA fiber several times to create a scaffolding disc, whereas, the successive splitting of the pyriform fibers in the gumfoot disc (Figure 8.3) indicates that the anterior spinneret rub
against the substrate once, going inwards from the periphery of the gumfoot disc. This also implies that the pyriform fibres are not completely solidified when secreted and that they coalesce to form thicker fibers, going inwards from the periphery of a gumfoot disc. Incidentally, cribellate spiders also use nanofibers for adhesion [65]. However, unlike in pyriform fibers, these nanofibers are drawn from the cribellum of the spiders by a setal comb on their legs [65]. Moreover, the cribellar fibers are solidified upon drawing, as is indicated by their uniform diameter, unlike pyriform fibers.

Figure 8.4| Sample preparation for adhesion measurements. Figure a shows the cobweb-weaving spider *Achaearanea tepidariorum* spinning a scaffolding disc on a nylon thread. The inset shows an optical microscope image of a scaffolding disc spun on a nylon thread (30 μm diameter). The black arrows point towards the nylon thread in both Figure a and its inset. Figure b shows a schematic of a typical cobweb. The black arrow points towards the flag used to collect individual gumfoot threads *with* the attached pyriform attachment disc (light green).

To determine the effect of architecture on attachment strength, the discs were peeled from a clean glass surface at a controlled rate, by pulling on the dragline silk/gumfoot silk thread perpendicular to the plane of the respective discs. Interestingly, scaffolding discs (for both cob- and orb webs) could not be induced to peel from glass by
pulling the underlying dragline silk vertically. Instead the dragline silk itself always failed before complete peeling of the scaffolding discs. This demonstrates that the adhesion force of the scaffolding discs is greater than the breaking force of MA silk (dragline silk), which is the strongest (highest breaking force) silk used in the web. Spiders were hence made to spin scaffolding discs on glass slides laid across a much thicker nylon thread (30 μm diameter) (Figure 8.4a), to test the discs’ maximum adhesive strength. To quantify the adhesion of gumfoot discs, gumfoot discs were peeled by pulling on the lower region of the gumfoot thread, which is composed of four MA fibers [59]. For sampling the gumfoot discs for adhesion measurements, the spider cages were layered with glass slides such that after the webs were spun, a flag, like that shown in Figure 8.4b was used to collect the gumfoot silk thread with the attachment disc undisturbed. Figure 8.5a shows the adhesion forces of the discs on glass. Scaffolding discs binds an order of magnitude stronger than gumfoot discs.

Figure 8.5| Adhesion measurements. Figure a shows the force required to separate a gumfoot disc and a scaffolding disc from a clean glass surface. Gumfoot disc adhesion is an order weaker than scaffolding disc adhesion. The force required to break a dragline silk (MA silk) thread is also plotted. A dragline silk thread breaks when it is pulled to peel a scaffolding disc (hence thicker nylon threads were used to peel scaffolding discs). Gumfoot discs were peeled using the gumfoot silk threads (four intertwined MA threads). In theory, however, gumfoot discs could be peeled using just one MA thread since the
breaking force of MA thread is almost twice the adhesion of gumfoot discs with glass. This indicates the scaffolding disc adhesion is much stronger than gumfoot disc adhesion. Figure b shows the energy of adhesion of a gumfoot disc and a scaffolding disc. Gumfoot discs adhere an order weaker than scaffolding discs. The energy of adhesion is determined by subtracting the strain energy contribution of nylon threads (while peeling scaffolding discs), and gumfoot threads (while peeling gumfoot discs) from the total work done in peeling the discs.

The total work done in peeling the discs can be calculated by integrating the area from the force-displacement results. The total work done involves work incorporated in stretching the thread (nylon in the case of scaffolding discs) and the energy expended in peeling the discs. The strain energy stored in nylon threads and gumfoot silk threads was independently determined from tensile stress-strain measurements and was subtracted from the total work done (calculated by integrating the area under the force-extension curve obtained from the disc peeling measurements) to calculate the work done due to peeling as shown in Figure 8.5b, for scaffolding discs and gumfoot discs, respectively. Scaffolding discs adhere much more strongly than gumfoot discs, as anticipated (The work done due to peeling comprises of stretching, separating, and breaking of pyriform fibers, as shown by high-speed videos taken during peeling, and should thus, in theory, be much higher than the thermodynamic work of adhesion of pyriform fibers).

The difference in adhesion cannot be solely attributed to the difference in the number of fibers in both the joints since in gumfoot joints, all the fibers are acting together while in scaffolding joints, the number of fibers sharing the load is only a fraction of the total number of fibers present (1550), which depends on the size of the deformation zone developed during peeling of the scaffolding joint. These observations
and results clearly demonstrate that spiders tailor-make the attachment discs to vary adhesion and customize function of thread attachments.

8.5 Discussion

The difference in adhesion energies caused by divergent architectures has major implications on locomotion and prey-capture by spiders. When an insect encounters an orb-web (which is also attached to different surfaces using pyriform discs spun in the staple-pin architecture), the hysteretic behaviour of the MA fibers, as they stretch and relax, account for majority of the energy absorbed by the web [131]. Also, when an insect is completely entangled, the weight of the insect is supported by the MA fibers, and hence the load is transferred to the attachment points of the web with the surface. Stiffness, strength, toughness, and viscoelasticity of the MA silk are key to the functioning of both orb and cobwebs. However, the excellent properties of MA silk can only be utilized for capturing prey if threads are firmly fixed to surfaces. Spiders accomplish this by creating attachment discs out of pyriform silk that have a ‘staple-pin’ architecture. The observation that the adhesion strength of these discs is higher than the breaking strength of MA silk has major implications for optimized web-engineering: the mass and velocity of the incoming prey are limited solely by the breaking strength of the strong MA silk and not by its attachment to surfaces.

In contrast, the gumfoot discs are attached very weakly to the substrate, which enables the gumfoot threads to detach easily and energy stored in the scaffolding silk to
suspend the insect helplessly in the air. A gumfoot thread is composed of four MA silk threads at its base [59]. During adhesion measurements, gumfoot discs could be easily peeled from a surface by vertically pulling the gumfoot thread attached to the discs. The adhesion force of a gumfoot disc is \( \sim \) 50\% the breaking force of one MA silk thread (~1/8\(^{th}\) the breaking strength of a gumfoot thread, since there are four MA threads in the lower region of a gumfoot thread).

To explain why the scaffolding discs bind stronger than the gumfoot discs, we use a classical tape-peeling model described by Kendall [132] and Hamed [133] where the peeling force \( F \) is related to the adhesion fracture energy \( G \) and physical parameters such as width \( w \), thickness \( d \), peeling angle \( \theta \), and modulus \( E \)

\[
\left( \frac{F}{w} \right)^2 \frac{1}{(2dE)} + \left( \frac{F}{w} \right) (1 - \cos \theta) - G = 0
\]  

(1)

There are several key predictions that are relevant in understanding the strength of the attachment discs. First, the peeling forces decrease with increase in peeling angle. Second, at low peeling angles, the peeling forces depend on the thickness, width, and modulus of the attached tape strips.

\[
\frac{F}{w} = \sqrt{2EdG}
\]  

(2)
However, at high peeling angle, the peeling force depends on only the width and is independent of the thickness and the modulus of the tape.

\[ \frac{F}{w} = \frac{G}{1 - \cos \theta} \]  

(3)

Figure 8.6 | Modelling the discs. Figure a shows a schematic of the simulated scaffolding disc. The length \( l_{sp} \) is 100 mm while the width \( w_{sp} \) is such that \( 6 \times w_{sp} = 36 \) mm. The inset shows how the peeling measurements were conducted. The nylon strand is pulled on perpendicular to the plane of the tapes while the load-extension behaviour is registered.
Figure b shows a schematic of the simulated gumfoot disc. The length $l_{rp}$ and the width $w_{rp}$ of the tape strips is equal to $l_{sp}$ and $w_{sp}$, respectively. All the six strips are firmly secured to the strand of nylon threads at the center of the circle. The insets on the left and right show the top and front views of the modelled tapes. Figure c shows the load-extension results obtained from the adhesion measurements of the simulated scaffolding discs. Circles denote the case with length $l_{sp}$ and width $w_{sp}$, squares: $l_{sp}/2$, $w_{sp}$, and triangles: $6w_{sp}$, $l_{sp}$. The inset shows a plot comparing the adhesion forces of simulated scaffolding silk with the simulated gumfoot disc, both using the same dimensions of the strip: $l_{sp}$, $w_{sp}$. Figure d shows the load-extension results obtained from the adhesion measurements of the simulated gumfoot discs. Circles denote the case with length $l_{rp}$ and width $w_{rp}$. Squares denote length $≈ l_{rp}/2$ and width $w_{rp}$, upright triangles: length $l_{rp}*3/4$ and width $w_{rp}/2$, and inverted triangles: width $w_{rp}$ and length $≈ 3mm$.

Equations 1-3 are valid for peeling of long elastic tapes and where the bending energy is constant during peeling. If plastic deformation is involved in the peeling, then the adhesive forces at high peeling angles may also depend on thickness and the modulus of the tape. Hamed and Gent [133] showed that, at high enough bend diameters, the bending contribution is negligible at higher peeling angles such that the equations derived for elastic tapes could be used at the $90^\circ$ peeling angles used in our experiments.

The scaffolding disc is then modelled by attaching a strand of nylon fibers on a clean glass surface using six strips of a stretchable tape (3M, MMM8884) perpendicular to the long axis of the strand (Figure 8.6a). Peeling of the tape strips is induced by pulling on the ‘peeling end’ of the nylon strand perpendicular to the plane of the tapes, at a fixed rate (inset, Figure 8.6a). The rate of pulling is kept fixed throughout the study because the fracture energy $G$, and hence the peeling force $F$, depend on the rate. The gumfoot discs are simulated by placing six strips of the same tape in a radial pattern, such that all the strips are securely attached to a strand of nylon threads at the center of the circular disc (Figure 8.6b). Here, peeling of the tapes is induced by pulling the nylon
strand perpendicular to the plane of the tapes, at a fixed rate. Figures 8.6c and 8.6d show the adhesion force of both geometries. For the same surface area of the tape, the ‘staple-pin’ geometry (mimicking scaffolding discs) requires a much larger force to detach than the dendritic geometry (mimicking gumfoot discs) (blue circles vs. green circles and blue squares vs. green squares, Figures 8.6c and 8.6d). The energy expended (area under the load-extension curve) is also higher for the ‘staple-pin’ geometry.

Peeling the modelled scaffolding disc involves deformation of the tape strips and their concomitant detachment from the glass surface. The detachment of the tape from the glass surface follows a near-zero degree peeling behaviour, with small gradual increase in peeling angle during further peeling. Consistent with Kendall’s observations (Eqn. 2), the thickness of the tape strips, d, affects the fracture energy of the simulated scaffolding disc (Figure 8.7a, solid blue squares).

![Figure 8.7](image_url)

**Figure 8.7** Dimension-dependence of peeling forces. Figure S3a shows the load-extension response obtained from peeling of simulated scaffolding disc. The length of the strips is l_{sp}, width w_{sp}, but the thickness is twice from Figure 8.6c. Change in thickness affects the peeling force. Figure b shows the load-displacement response obtained from peeling of simulated gumfoot discs. For filled green squares, the length of the strips is l_{rp}, width w_{rp}, but the thickness is twice from Figure 8.6d. Thickness has no effect on peeling.
force here. For filled green diamonds, length is $l_{rp}$, thickness is same as in Figure 4d, but
the width goes from $w_{rp}$ at the proximal end to 1 mm at the distal end, with a linear
gradient. This gradient best represents the gumfoot discs.

Detachment of simulated gumfoot discs, however, initiates as a near-ninety
degree tape-peeling, the peel angle reducing only slightly during further peeling, and is
explained by Equation 3. (The testing method for both the modelled discs involved slight
changes in the peeling angle during the course of peeling. This was done to simulate the
real-life scenario and also the method used to test the natural attachment discs). The
radial peeling of six tape strips of width $w_{rp}$ each was observed to be equivalent to
peeling of a single tape strip of width $6*w_{rp}$. Each curve shows a ‘fracture-initiation
region’, followed by a ‘fracture-propagation region’. As explained in Equation 3, the
propagation region scales with the width of the tape: a tape of width $<w_{rp}$ shows a
propagation region of smaller magnitude (inverted triangles, Figure 8.6d), while a
shorter tape of the same width has a propagation region of the same magnitude (squares,
Figure 8.6d). Successively reducing the length of the tape, keeping width constant,
results in the extreme case where just the initiation region is observed: fracture-
propagation is absent (green triangles, Figure 8.6d). Also, varying the thickness of the
tape has no effect on radial peeling, unlike in the staple-pin case, consistent with
Kendall’s observations (green solid squares, Figure 8.7b). The slight increase in the
propagation regions of all the curves is explained by the small reduction in peeling angle
during peeling (Equation 3).
To accomplish peeling of the modelled discs, a nylon strand of breaking force >> 200N was used. In reality however, MA silk thread breaks before peeling of the scaffolding discs. This is simulated by using a thinner strand of nylon threads (breaking strength ~ 60N). Using this strand, the simulated gumfoot disc still peels completely but an attempt to peel a simulated scaffolding disc instead causes fracture of the nylon strand before the disc detaches. These simulation results clearly demonstrate the design principles employed by spiders to make strong versus weak joints using the same material.

A notable feature in the scaffolding discs is their high aspect ratio. The lengths of the pyriform fibers are 250 times the diameter of the secured MA silk thread (~ 1000μm vs 4μm), which seems to suggest that a lot of ‘expensive material’ used to spin pyriform fibers is wasted, and that pyriform material could either have been saved or used to cover a longer length of MA silk thread using shorter pyriform fibers. We tested these conditions, with the nylon strand and the stretchable tape, to show why longer lengths of pyriform fibers are crucial to create strong joints (Figure 8.6c). Keeping the width of each strip constant \(w_{sp}\), if the length of each strip is reduced to \(l_{sp}/2\) (so that the volume of tape is essentially halved), the adhesion force is reduced by almost 50% (blue squares vs. blue circles, Figure 8.6c). In contrast, using the same volume of the material to produce wider strips \(l_{sp}/6\), where length is reduced to \(6*w_{sp}\) (blue triangles, Figure 8.6c) reveals an interesting behaviour: even though the length of the nylon strand covered by the tape to immobilize it on the glass surface is now much longer, i.e. \(l_{sp}\) compared to the previous value of \(6*w_{sp}\), the force and energy required to detach is smaller than 50%.
Interestingly, this value is even lower than when 50% of the material (length of each strip \(l_{sp}/2\)) was used (blue triangles vs. blue squares).

During staple-pin peeling, the tape strips have to be deformed for the fracture to initiate and propagate. In the first case (width \(w_{sp}\), length \(l_{sp}\)), peeling requires higher force to deform and detach the longer strips of length \(l_{sp}\). However, in the third case (width \(l_{sp}/6\), length \(6*w_{sp}\)), much smaller force is required because the length to be deformed and detached is only \(l6*w_{sp}\). This might explain why spiders use long fibers to make the scaffolding disc.

Lastly, we discuss why spiders employ two different architectures for making strong and weak joints when one architecture employing different amounts of material could instead be used to change the adhesion force of a joint. Varying the amount of material in a ‘dendritic’ disc does not change the force required to peel the disc (Figure 8.6d, green circles and green squares). In the actual gumfoot discs, fibers in contact with the surface are mostly at the periphery of the disc and become successively thinner going towards the periphery (Figure 8.3), which is most accurately modelled by using tapered strips of tape represented by green diamonds in Figure 8.7b. Varying the amount of material in a ‘staple-pin’ disc does change the force required to peel the disc, so it could potentially be used to make both strong and weak joints. However, spiders do not use the same architecture because, in addition to the major requirement that scaffolding discs should adhere strongly and gumfoot discs should adhere weakly, there is one more requirement for the gumfoot discs to act as an efficient trap: discs should release with ease only after being agitated by an insect. Gumfoot threads are spring-loaded traps that
are always held in tension. These traps are triggered when agitated by a walking insect. An ideal trap acts spontaneously upon triggering by prey, but not minor perturbations of the web due to wind or even the spider moving through its own web. We hypothesize that the dendritic architecture facilitates an ideal trap-like behaviour for the gumfoot discs. The initial over-shoot region acts as a trigger, going past which the propagation region slopes downwards (Figure 8.7B, green triangles). Once the agitation force caused by the insect surpasses the initial over-shoot, the gumfoot discs rapidly release with ease, suspending the insect helplessly in the air for the spider to subdue. The force profile during peeling of a staple-pin disc slopes upwards, which is ideal for developing a strong joint, since it needs to be “strain-hardening”, but it does not fulfil the trigger requirement of an ideal spring-loaded trap.

It follows from our observations of the actual discs and our simple yet effective modelling of attachment discs that architecture does indeed play a key role in tuning not only the adhesion strength but also the release ‘behaviour’. The same protein-toolkit
Figure 8.8| Evolutionary convergence in using architecture to mediate attachment disk function. Orb-weaving spiders are the evolutionary ascendants of cobweb-weaving spiders. Figure shows a staple-pin attachment disc spun by the orb-weaver *Larinioides cornutus*, architecturally similarly to the scaffolding disc spun by cobweb-weavers (Figure 1a). This disc is used by orb-weavers to attach their webs and draglines to different surfaces to different surfaces, thus facilitating prey-capture and locomotion, respectively. The chemical composition of this disc has been shown to be different from that of scaffolding disc of cob-weavers, but the architecture is identical. This disc, like the scaffolding disc of cob-weavers, binds stronger than the breaking force of an MA fiber, which has important consequences in web-engineering, as explained in the text. The scale bar is 100 µm.

(pyriform silk fibers) is utilized by cobweb spiders to create two distinct attachment discs – a staple-pin architecture that adheres firmly and a previously described dendritic architecture that detaches easily. Interestingly, the evolutionary ascendants of cob-
weaving spiders, the orb-weaving spiders, also use ‘staple-pin’ architecture to firmly attach their orb-webs to different surfaces, similar to how cob-weavers attach their scaffolding sheet to a variety of surfaces (Figure 8.8). The chemical composition of the proteins in the pyriform silk for orb-weavers has been shown to be different from the cob-weavers [134]. However, the adhesion forces are very similar, emphasizing the importance of the architecture rather than the differences in the chemical composition of the pyriform silk.

The adhesion forces in these studies were measured on glass and this is a simplified perspective considering that the spiders use surfaces including wood, leaves, and even concrete. Based on our field observations, it appears that scaffolding joint adheres much stronger than the force required to break the MA silk thread. However, the gumfoot joint adhesion depends on the surface, for example, they fracture cohesively on a clean glass surface (Figure 8.9) but fracture adhesively on an aluminium surface. The successive bifurcation of glue threads (Figure 8.3) would either facilitate breaking or precipitate detachment through peeling depending on the surface.
Figure 8.9 | Post-peeling scenario. Figure shows optical microscope images of joints post-peeling. Figure a shows a scaffolding joint while Figure b shows a gumfoot joint. The black arrows in Figure a and its inset point towards the peeling zone of the scaffolding joint spun on a nylon thread. Pyriform fibers peel a certain distance followed by breaking, as is shown in the inset. The scale bar in Figure a and its inset is 100 µm and 25 µm, respectively. Figure b shows a gumfoot joint post fracture. Gumfoot joints are bound to a surface with the help of fibers that are much finer than in scaffolding joints, as has been explained in the text. These fibers peel a much shorter distance and then break, on account of being very fine. The arrows point towards darker regions of the joint post-peeling which indicates the broken ends of these finer pyriform fibers. The scale bar in Figure b is 50 µm. Gumfoot joints can detach by complete peeling too depending on the surface.

In summary, the cobweb-weavers and orb-weavers apply pyriform silk in different ways to achieve either a very strong attachment of the discs to hold the dragline permanently to substrates or controlled attachment points that release rapidly when contacted by prey. Our models show that this variation in adhesion strength and behaviour does not require specialization of the chemistry in the glues, but is clearly controlled by architecture. It is now recognized that the spinning process is very important in achieving high tensile strength, extent of super-contraction, and the excellent properties of silk [96, 135, 136]. Here, we emphasize how spiders also use the architecture of attachment to control the strength of the adhesion. These results provide design principles of using synthetic adhesives to fabricate tunable structural adhesives by using the known chemistry available today.
Spiders employ clever behavioral strategies combined with almost invisible custom-made adhesives for locomotion and prey-capture. The adhesives used in spider webs have evolved over millions of years into a class of natural materials with outstanding properties. We show here that the ‘magic’ lies not in the composition but in the structure and architecture of these adhesives, over different length scales.

The adhesive produced by modern orb-weaving spiders to capture prey (viscid glue) is laid on a pair of extensible axial silk fibers as micron-size glue droplets that are composed of a mixture of salts and polymeric glycoproteins. By stretching individual droplets, we show that the viscid glue behaves like a viscoelastic solid and that the elasticity is critical in enhancing adhesion caused by specific adhesive ligands by over two orders of magnitude. The viscoelastic solid nature of the glue imparts time-dependent adhesion to the glue drops. At rapid extension rates, the adhesive forces are dramatically enhanced due to high viscous effects, making it easier for the capture silk threads to hold on to fast flying insects when they initially impact webs. We show that the structure of
the glycoproteins at the nanolevel, which makes it elastic, enhances the overall adhesion of the glue by two orders of magnitude in comparison to capillary forces of the droplet itself, thus putting to rest the old notion of the adhesive being viscous. We compared the single drop stretching results with whole thread adhesion results. The forces obtained from whole thread adhesion measurements depend on the contribution of the glue adhesion and that of axial silk extension. We developed an energy model to separate the two contributions. A good agreement was observed between whole thread measurements and single drop stretching results. These results have been described in detail in Chapter IV.

Glycoproteins, according to previous studies, make up only 10% of whole droplet volume. The majority component of glue drops are salts and the water that the salts sequester from the atmosphere. Interestingly, these salts, that are present in large quantities in the web, are nutritionally and physiologically essential for the spider. Intuitively, one would think that the main function of the salts is not just to sequester water, and that the salts play a major role in adhesion of the capture threads. We show that the salts are necessary to solvate and soften the glycoproteins and upon removal of the salts the threads do not adhere. We do so by washing whole threads with de-ionized water. Salts, being water soluble, are washed away but no glycoproteins are removed. Whole thread adhesion measurements and energy model developed by us show that washed capture threads do not adhere, nor do they interact with water. Solid-state NMR measurements support our measurements. For enhancing the signal-to-noise ratio of the glycoproteins in the solid-state NMR measurements, spiders were fed with C-13 labeled glucose. We also hypothesize that salts trigger the phase separation with each glue
droplet, and facilitate the development of glycoprotein in two phases: the core, which helps as an anchor and maintains cohesion within the drop, and the shell, that establishes contact and is the adhesive component. The microstructure of each glue droplet helps it be an effective reversible adhesive. Chapter V covers this study in detail.

Now that we realized that salts are an essential part of the capture silk threads, we looked at another interesting spider adhesive that does not have most of these salts. This system is the adhesive system of the cobweb-weaving spiders (gumfoot glue), the evolutionary descendants of orb-weaving spiders. Upon comparison, it was observed that, in spite of being produced in homologous aggregate glands, the gumfoot glue behaves like a viscoelastic liquid, as opposed to the viscoelastic solid nature of the viscid glue. Moreover, the gumfoot glue is largely resistant to humidity; elasticity and adhesion are constant across variation in humidity and there is weak volume-dependence. Viscid glue, however, is highly humidity-sensitive. The glue expands an order of magnitude and demonstrates a monotonous reduction in elasticity under increased humidity, while glue adhesion optimizes at intermediate levels of humidity. We hypothesize that the above-mentioned properties of the gumfoot glue are due to the presence of aqueous peptides. Solid-state NMR measurements will be done to investigate this. Chapter V1 discusses these results.

Despite the dramatically different properties of the glues themselves, the utilization macro-architecture of the glue remains conserved across generations: Beads-on-a-string (BOAS) architecture. To understand why spiders use these adhesives in a BOAS structure in their prey-capture threads, we employed their adhesive thread building
strategy to produce functional micro-threads that are similar in the micro-structure (BOAS) and adhesive properties to spider capture silk. Using these functional micro-threads, we show that the BOAS structure adheres more than a cylindrical structure during contact (collision of insect with the thread) and during separation (when an already trapped insect tries to rescue). This might explain why the BOAS structure is so prevalent throughout the evolutionary tree of spiders. Chapter V11 discusses this in detail.

All the amazing properties of spider silk: the adhesion of capture threads, the strength, and stiffness of the major ampullate threads would be waste if the webs spun by spiders could not stick well. Spiders spin attachment discs out of pyriform threads to attach their major ampullate threads (radial threads in orb webs, and scaffolding silk as well as gumfoot threads in cobwebs) to different surfaces. We discovered how just by changing the architecture of the attachments discs, spiders tailor the adhesion of these pyriform attachment discs to make strong or weak joints. Spiders’ three-dimensional cobwebs can target both walking and flying prey. While the scaffolding silk and supporting framework can sometimes entangle flying insects, gumfoot silk threads function as spring-loaded traps that pull walking prey off the ground and into the web. The scaffolding silk needs to withstand the impact of the prey and support the whole cobweb, including the gumfoot silk. In contrast, the gumfoot silk needs to easily detach from the substrate at its gluey base when contacted by prey. We show that spiders accomplish these divergent demands in mechanical performance by creating silk attachment discs of two distinct architectures using the same pyriform silk secretions. A ‘staple-pin’ architecture is used to firmly attach the scaffolding silk to the substrate and, a previously unknown, ‘dendritic’
architecture is used to weakly attach the gumfoot silk to the substrate. By modifying the architecture of the pyriform silk secretions spun by cobweb weavers, the gumfoot attachment discs adhere weakly, triggering a spring-loaded trap, while the scaffolding discs adhere so strongly that the major ampullate scaffolding threads break instead of detaching. We show how Kendall’s tape-peeling model describes the differences in peeling force for these two architectures and how these design principles can be incorporated into synthetic attachments. Discovering how spiders use silk attachment to functionalize their webs therefore reveals important design principles for controlled adhesion. Chapter V111 discusses these results in detail.

This research, thus show the importance of structure at different length scales in influencing adhesion and shall inspire future efforts directed towards tunable adhesives.
BIBLIOGRAPHY


