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Modulus Enhancement of Hydrogels of Squid Ring Teeth Proteins

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Honors Research Project

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Honors Abstract Addendum

The mechanical properties of the esoteric squid ring teeth (SRT) proteins, or suckerins, were analyzed. Hydrogels were made by mixing heated gelatin with crushed SRT proteins in solution, which was reacted with ammonium persulfate (APS) and Tris(2,2'-bipyridyl)dichlororuthenium hexahydrate (Ru). The compression moduli of the gels were determined using a contact mechanics method[1] derived from the Johnson-Kendall-Roberts theory[2]. In this study, the native SRT proteins were used to generate some preliminary results. The addition of native SRT proteins to a gelatin can increase the Young's modulus of the formed gels. Since proteins derived from SRT, i.e., suckerins, were found to be one of the strongest proteins discovered to date, they can be used as an additive that can add strength and flexibility to applications such as synthetic bone, cartilage, or tendon tissue, textiles, and specialty polymers.

Executive Summary

Purpose

Squid ring teeth (SRT) are a ring of protein found in the suckers of squid tentacles which look like a ring of teeth. These proteins are hardened like teeth, yet they behave like a thermoplastic. SRT have the potential to be used in a myriad of applications, but more research is required to find the most efficient ways of collecting and processing SRT. One application considered for the utilization of SRT is in hydrogels, which are used in various fields, but primarily in the medical and pharmaceutical fields. SRT can be used to increase the mechanical properties of hydrogels.

Results

Once SRT material was broken down using a mortar and pestle, it could be dissolved in a 5 wt% acetic acid solution. After the SRT solution had been reacted with ammonium persulfate (APS) and a ruthenium based catalyst (Ru) in the presence of a 500 W halogen lamp, it was mixed with a heated gelatin solution at various ratios and cooled in a refrigerator to form a series of gels that could be tested. These gels underwent a contact mechanics test where a lens was slowly pressed into the gel and then slowly removed until the gel and lens had fully separated. The force on the gel and the position of the lens were recorded in order to calculate the modulus of each gel.

Based on these observations, the addition of SRT can increase the modulus of the gel. Gelatin hydrogels that were 4.5 wt% solid were created by combining gelatin and SRT solutions. The solid phase wt% of SRT was experimentally varied from 0-10 wt%. The hydrogel peak modulus was observed at ~6-8 wt% SRT to gelatin.

Conclusions

SRT is capable of raising the modulus of gelatin. The addition of SRT to gelatin increases the strength of the hydrogel up to an SRT to gelatin ratio of about ~6-8 wt%, after which the increased strength begins to decrease. Regardless, there is an increase in modulus in all gels that have SRT from the gels that do not up to 10 wt% SRT to gelatin. Hydrogels that need increased strength and processability could look to SRT as an alternative additive, which would be more environmentally friendly than its hydrocarbon based alternatives.

Discussion

Researching SRT has taught many lessons and provided for many learning opportunities. Skills such as reading journal articles, designing and carrying out experiments, keeping a lab notebook, data collection and analyses, and writing a technical report were all employed, all of which are important to chemical engineering. There were opportunities to find the right materials and chemicals to use and some intricate apparatuses were created.

In general, this research has encouraged me to generate my own methods to approach a project that has no bounds whatsoever. As SRT are largely unknown to most people, there is no preconceived starting point for an application. The project demanded that new pathways be invented. This helped me to be creative and independent with my experimental design, although I had tremendous help and I asked countless questions.

Some synthetic materials used in the medical and materials science fields can be rigid, difficult to mold to fit a shape, are often made of petroleum based polymers, and, especially in the medical field, have difficulties associated with being compatible with human tissue. Additionally, the petroleum used to make most polymers is a non-renewable resource. Along with the great push to find renewable energy pathways through solar panels and windmills, there has been a renewed emphasis on recycling and interest in using natural materials. Squid ring teeth (SRT) protein is a natural material that has a thermoplastic behavior that allows them to be molded in a facile manner, as well as a relatively high Young's modulus which provides the material with strength.

Recommendations

The process of finding a functional plan of attack was long and testing was not completed. More variables of the SRT and gelatin mixture should be tested. The order of when the SRT is reacted with APS and Ru should be adjusted and full tests should be run on each iteration. More research should go into creating a hydrogel out of the SRT with APS and Ru alone and without the use of gelatin and what concentrations of each chemical should be used. Care could be taken to find the best method of providing light to the Ru catalyst, whether it is by a 500W halogen light or by an ultraviolet light source.

If your research involves something as esoteric as SRT, read literature all day everyday for a couple of weeks before you start to design your experiments. As you learn more, you realize that the work you are completing is irrelevant to your specific task at times. Reading literature gives a solid background that will support all future endeavors. Beyond that advice, it is important to immerse yourself in the project that you do. Especially in a technical degree such as engineering, it is difficult to find a creative outlet for your knowledge, and this research project is an opportunity to be as imaginative as possible and to create something that embodies the things you have learned, the experiences you have amassed, and the skills you have acquired.

Introduction

Have you ever heard of squid ring teeth? They are small rings of protein that can be found in the suckers on squid tentacles and they look like sharp teeth. Many squid species including *loligo vulgaris and dosidicus gigas* use these miniscule teeth as a tool to latch on to prey[3,4,9]. However, the proteins that make up the squid ring teeth (SRT) are more fascinating for their material properties than for their functional properties. Although SRT are made of protein, they are hard like tooth enamel or an insect's exoskeleton and have relatively high mechanical properties for a protein such as modulus of elasticity[4]. Furthermore, SRT exhibits a thermoplastic behavior in which it can be divided in two and then reconnected with a little heat and pressure. Being a protein, SRT can be artificially synthesized using RNA sequencing methods[3]. These properties of SRT are helping it become a popular material for biotechnical and material science research.

There is a wonderful opportunity when new materials are discovered to apply them to new technologies. A significant portion of the research done on SRT was to uncover a viable application for this burgeoning topic. SRT is getting a lot of attention in the biological and life sciences areas as researchers have mapped the genomic data of SRT and learned to synthesize it using RNA sequencing[3]. Without access to the equipment necessary to carry out RNA sequencing, the SRT had to be analyzed using physical and chemical methods. Hydrogels, especially the mechanically strong hydrogels, are a class of materials that are gaining importance, so it was decided that the effects of adding SRT to gelatin would be tested. This provided the application pathway necessary to develop an experiment.

To determine the strength enhancement of hydrogels with SRT proteins incorporated, the moduli of the gels containing different amounts of SRT proteins were evaluated. The moduli of the hydrogels were determined using a contact mechanics approach[1] based on the Johnson-Kendall-Roberts theory[2]. A lens was brought into contact with each gel being tested and was then pressed down and pulled up until the gel and the lens fully separated. The force of the lens on the gel and the position of the gel were recorded, and these values were used to calculate the modulus of each gel. The addition of SRT was able to increase the modulus of the gelatin meaning SRT can be used to strengthen hydrogels.

Background

Research on SRT is still in its early stages. A large portion of the work that has already been done is to elucidate the genetic makeup and structure of the proteins that come together to provide SRT to the suckers found on the tentacles of various squid species such as *loligo vulgaris* or the common squid. Recently, studies have shifted to understand the mechanical and chemical properties of the protein which demonstrates properties of the highest synthetic polymers while being made completely of proteins and lacking any mineral phase[4].

Hiew and Miserez have done a phenomenal job of chronicling the advancements in SRT technology. SRT were initially reported to exist by French naturalist Alcide D'Orbigny in the 1850's[6]. Chitin is a natural hardening agent that can be found in insect exoskeletons. Chitin was thought to be the reason for the mechanical properties of SRT[4,6]. When SRT was tested for chitin content using acid hydrolysis, it was surprisingly found to have no chitin[4,6]. Additionally, SRT lacks a mineral phase[4,6]. SRT was found to dissolve in a 5% acetic acid/6 M urea solution, which is useful because most naturally hard materials are resistant to strong denaturing solutions[4,6,11].

A crucial foundation was laid when researchers fully characterized the genomic data that can produce SRT. This led to the mapping of SRT amino acid sequences. The SRT are mainly made of two modules: one being rich in the amino acid alanine (Ala) and having similar characteristics to spider silk, the other being rich in the amino acids glycine (Gly), tyrosine (Tyr), and leucine (Leu)[4]. The silk-like proteins of SRT came to be called “suckerins” and their structure was defined as having strong β-sheets connected by amorphous networks[4]. The strong mechanical properties of SRT come from the hydrogen bonding localized to the β–sheets that form matrices that make up the SRT proteins[6]. These β–sheet matrices form to have dimensions of 3-3.5 nm in length and 2.4-2.6 nm in width, which means the length is made of ~8-10 amino acids, and the width is about 5 strands[6].

Recombinant SRT has already been created that can be formed into various functional shapes such as nanopatterned surfaces and photo cross-linked films that exceed the mechanical properties of most natural and synthetic polymers[3]. Much existing work that involves SRT has isolated certain SRT proteins called “suckerins” and has transcribed their DNA to create synthetic biomaterials out of it not only to isolate each protein and test each one, but also to have a significantly larger amount of material than can be removed from an actual squid. The present work was done without any RNA sequencing equipment.

An important mechanical property is Young’s modulus also known as elastic modulus. It is a measure of the amount of strain on an object in proportion to a tensile stress on an object. The dry elastic modulus of SRT has been calculated to be 6-8 GPa and the hydrated elastic modulus is 2-4 GPa[7]. For reference, a common plastic has a modulus of 2 GPa, and bone has a modulus of 18 GPa[8].

SRT is capable of undergoing a reversible solid to melt phase transition, which allows for it to be shaped into various configurations. This is due to SRT having a fairly low glass transition temperature, an indication of the temperature at which a polymer goes from a glassy crystalline structure to a more elastic semi-crystalline structure or vice versa. SRT was found to have a T_g of 32°C[9]. The SRT is structurally stable at temperatures up to 220°C. The amorphous module of the SRT exhibits viscous properties below the glass T_g of the β-sheet module[10].

Dynamic mechanical analysis (DMA) is a common way of determining the viscoelastic properties of a polymer. Polymers can behave as a viscous material that flows and loses energy, while simultaneously acting like an elastic material that stores energy. DMA returns a loss and storage modulus that corresponds with these behaviors. The ratio of the moduli is called tanδ. DMA of 18 kiloDalton SRT proteins showed them to have a storage modulus of ~1 GPa and a loss modulus of about 50 MPa which is comparable to high density polyethylene[11]. This means that SRT behaves more like an elastic material than a viscous material and would have a tanδ of ~.05.

A group has gathered gold nanoparticles onto recombinant SRT substrates. The high amount of Tyr in one of the structural modules of the protein provides for a phenol group for electron transfer between the substrate and gold nanoparticles[12].

It was decided the research would follow the path of adding the SRT to gelatin to produce hydrogels with increased modulus. Methods of synthesizing SRT hydrogels were considered. The Tyr of the amorphous sections in SRT can readily form crosslinks into di-Tyrosine which creates a crosslinked material out of the SRT[13]. A Ruthenium (Ru) based catalyst system was used with photo-crosslinking by Ding's group in order to create hydrogels out of suckerin-19[13]. The crosslinked suckerin was found to not be a cytotoxin[13]. Suckerin-19 was found to dissolve in a 5% acetic acid (AA) solution and was crosslinked using the Ru catalyst along with ammonium persulfate (APS) as an initiator[13]. APS concentrations of 0.1 – 2.5 x 10^{-3} M were capable of producing suckerin gels[13]. Light exposure time using a 500 W halogen bulb for photo-crosslinking could be as low as 5 s in order to carry out total crosslinking[13]. These gels were created from 4 wt% suckerin-19 solutions[13].

In order to quantify the force of adhesion of the gel, equations from the Johnson-Kendall-Roberts (JKR) theory were employed[2]. The JKR theory is a method of determining the force of attraction of two materials based on the interfacial geometries of the materials. As testing went on, it was understood that SRT on its own is not an impressive candidate to be an adhesive, and so practices from previous research that employed JKR equations were used[1]. A relation between load, P, and contact radius, a, is given in the following equation with R being the radius of curvature of the employed lens and K being a constant whose derivation will be described shortly.

$$\frac{a^{3/2}}{R} = \frac{1}{K}\left(\frac{P}{a^{3/2}}\right)$$

The constant K is derived from the Poisson's ratios of the two materials, ν_1 and ν_2, and the Young's modulus of both materials, E_1 and E_2. The Poisson's ratio is the ratio of perpendicular contraction to parallel elongation of a material when a tensile stress is applied to a material. The equation for K can be seen below.

$$\frac{1}{K} = \frac{3}{4}\left(\frac{1-\nu_1{}^2}{E_1} + \frac{1-\nu_2{}^2}{E_2}\right)$$

Finally, the contact radius, a, can be calculated from the following equation relating linear deformation, δ, and radius of curvature, R.

$$a = (\delta * R)^{1/2}$$

From these equations, it is possible to calculate the Young's modulus of a material if the displacement, radius of curvature, load, and Poisson's ratio are known. All of these can be measured or are readily available other than the Poisson's ratio of the hydrogel, which is assumed to be 0.5, a common value of the Poisson's ratio and roughly similar to most hydrogels.

Experimental Methods

Initially, there was uncertainty of how to obtain SRT. After searching local supermarkets, SRT was found on the squid tentacles and calamari that can be purchased in a frozen seafood medley to cook and eat. SRT was extracted from these squid tentacles underneath a microscope using tweezers at first until squids with larger SRT were made available. This method is illustrated below in Figures 1 and 2.

Figure 1 and 2: *Left, a researcher collects SRT from a squid tentacle. Right, SRT as they sit under a microscope, with a fingertip as reference. Both figures show the difficulty in obtaining large amounts of SRT.*

These SRT were then broken down in a mortar and pestle, sometimes after freezing them in liquid nitrogen, in order to increase surface area to help the SRT dissolve in solution. Various solutions were tested, but a 5 wt% acetic acid solution was settled on due to its ease to create. The concentration of SRT solution was set at 10 mg/mL, and solutions were kept in a refrigerator (RCA RFR321-FR320/8 IGLOO Mini Refrigerator, 3.2 ft^3). The SRT solution was reacted with APS in the presence of the Ru complex. The Ru was light-sensitive and so was kept in a vial covered in aluminum foil and care was taken to quickly replace the lid on the vial when it was not being used. The Ru concentration for testing was 5×10^{-5}, and APS concentration was 5×10^{-4}. The volume ratio of SRT solution to Ru solution and APS solution was 18:1:1[12]. This reaction was done in the presence of light from a 500 W halogen bulb (Bayco 500 W, 120 V, AC) on a stirring plate (VWR Microplate Shaker, VWR International) stirring at 500 rpm. This initial reaction begins the crosslinking of SRT material, which would help form a hydrogel with gelatin. The gelatin was heated on a hot plate (Corning 4x5" Top PC-200 Hot Plate) till ~60°C. The reaction setup can be seen below in Figure 3 and a closer view of the vial within its foam containment can be seen in Figure 4.

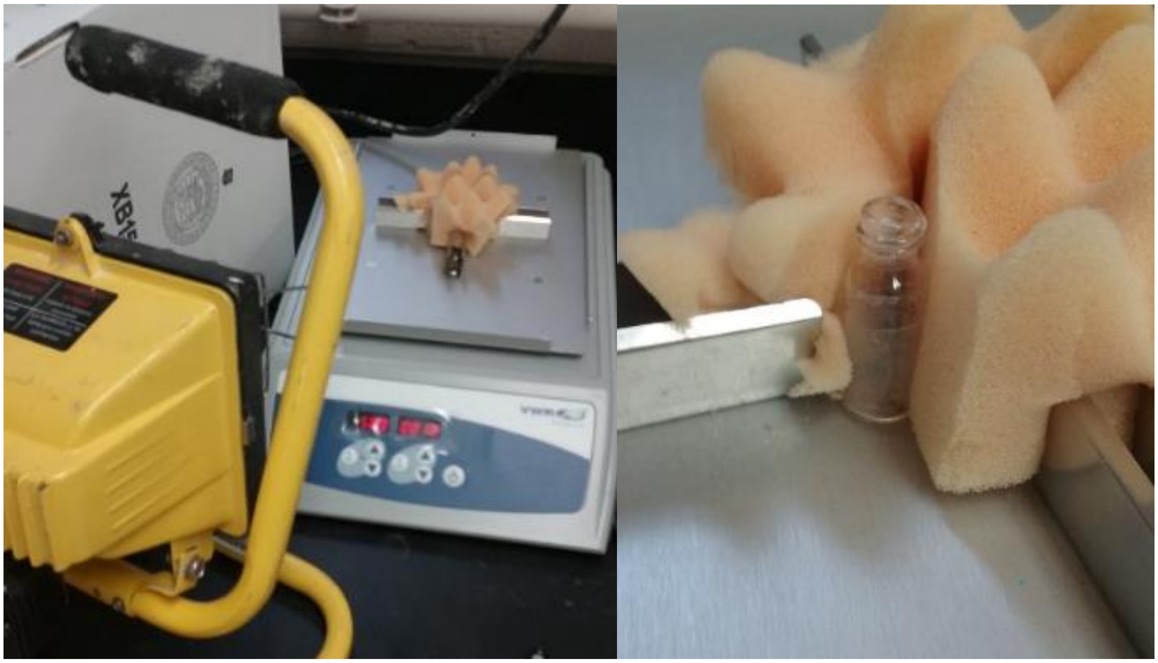

Figure 3 and 4: *Left, the setup where the reaction of SRT solution with APS took place in the presence of a Ru catalyst with a 500 W work lamp shining on it. The stir plate ensured that the reacting materials would be well mixed and the reaction would be homogeneous throughout the vial. Right, the vial was placed in the foam to keep it contained without damaging the vessel, yet positioned so that the 500 W light could reach it directly.*

Small vials were filled with the pre-treated SRT solution and DI water so that there would be 150 μL in a range of SRT solution to DI ratios from 0 to 1. Then, 150 μL of heated gelatin was added to each SRT and DI water solution in order to form a hydrogel with a volume of 300 μL. The resulting hydrogel was 4.5 wt% solids in solution. These vials, which can be seen in Figures 5 and 6 below, were kept in a refrigerator and care was taken to keep them hydrated.

Figure 5 and 6: *Left, the trays that the SRT hydrogels were created and stored in. These were detachable which made testing easier. Right, the hydrogel trays were placed in a container whose bottom was covered with water to help keep the gels hydrated. Despite efforts to keep the gels hydrated, the gels still dried out, causing difficulties in comparing tests.*

The hydrogels then underwent a compressive load test, which was carried out under the ambient conditions, with humidity adjusted using a humidifier. A glass capillary tube was heated and bent into the form of an “L” and then was attached to a glass slide that served as a cantilever. The end of the capillary tube was melted to form a hemi-spherical indenter, which was further modified with a hydrophobic silane, octadecyltrichlorosilane (OTS). The OTS modification was done to reduce the wettability of the indenter tip, due to the interaction with water in the hydrogel. In most cases, the water contact angle on the OTS modified glass was ~90°. A silicon wafer was attached to the shorter end of the capillary tube to assist with determining the distance that the indenter travelled. The gel was placed on a scale (Denver Instruments, Pinnacle Balance series) underneath the point of the capillary tube and light from a microscope illuminator (StockerYale Imagelite Lite Mite Series) was shone on the silicon wafer through a microscope (Infinity, Boulder, CO) attached to a video camera module (Sony CCD Video

Camera Module XC-75) with a 4x/0.10 objective. As the capillary tube was lowered using a Parker Hannifin Daedal 4602 Ball Bearing Positioner, the force on the scale, and therefore the interaction between the indenter and the gel, was measured every 5 seconds. Additionally, the position of the silicon wafer was recorded every 20 seconds. This set up can be seen in Figures 7 and 8 below. Refer to Figure 8 to see the silicon wafer clearly.

Figure 7: *The set up of the contact mechanics test showing the Infinity microscope and objective to the left, with the Parker Hannifin positioner to the right. The bent capillary tube can be seen in the middle above a stage for the gels made of Petri dishes atop the scale. The bent capillary tube can be seen more clearly in Figure 8.*

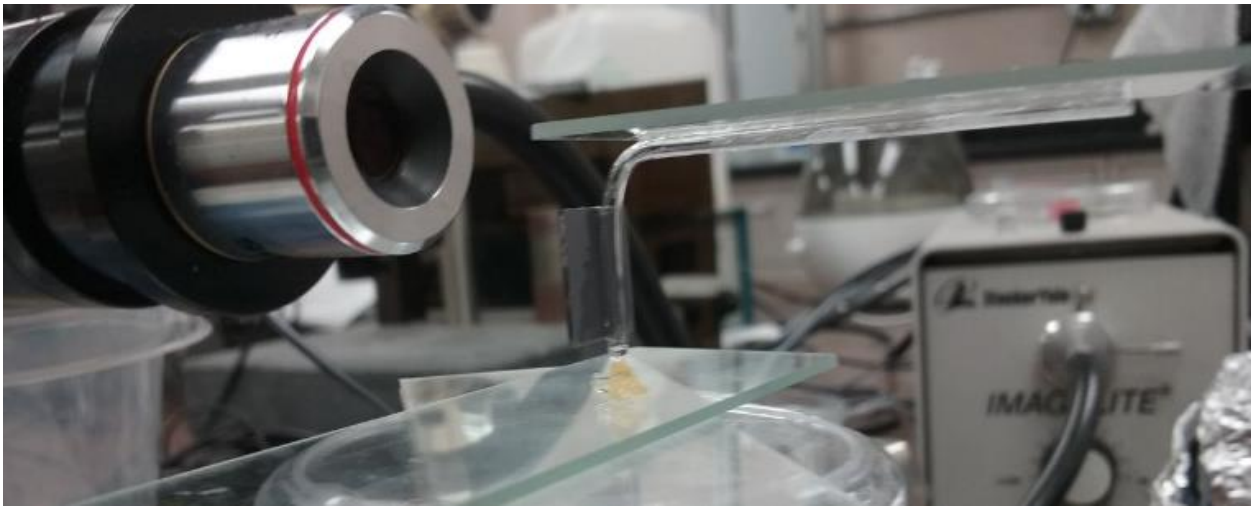

Figure 8: *A close up of the bent capillary tube apparatus. The objective can be seen to the left at the end of the Infinity microscope. The Imagelite microscope illuminator can be seen to the right in the background. An SRT hydrogel sample can be seen in the middle, as well as the back of the square silicon wafer attached to the capillary tube.*

The position of the silicon wafer provided the displacement of the gel surface due to the indenter.

An image of the capillary tube tip was used to calculate the radius of curvature which was determined to be 564 microns. Together with the radius of curvature of the indenter, the displacement was used to calculate the contact radius of each positioning of the indenter. With this data and the load information, the modulus of each gel could be tested.

Data and Results

SRT are capable of increasing the Young's modulus of gelatin. This statement holds true for all gelatin that had SRT material added to it. The added SRT gives peak performance when it is about 6-8 wt% of the overall SRT-gelatin makeup.

The deformation of the gel by the indenter at each recorded load measurement was used to calculate the contact radius. The deformation was recorded using the video recording module connected to the Infinity microscope. After one reference image was captured using Yawcam video capture software, the deformation could be measured by seeing how far a deformation in the silicon wafer moved between two recorded images using ImageJ software to measure the number of pixels between two points on a computer rendered image. This is illustrated in Figure 9.

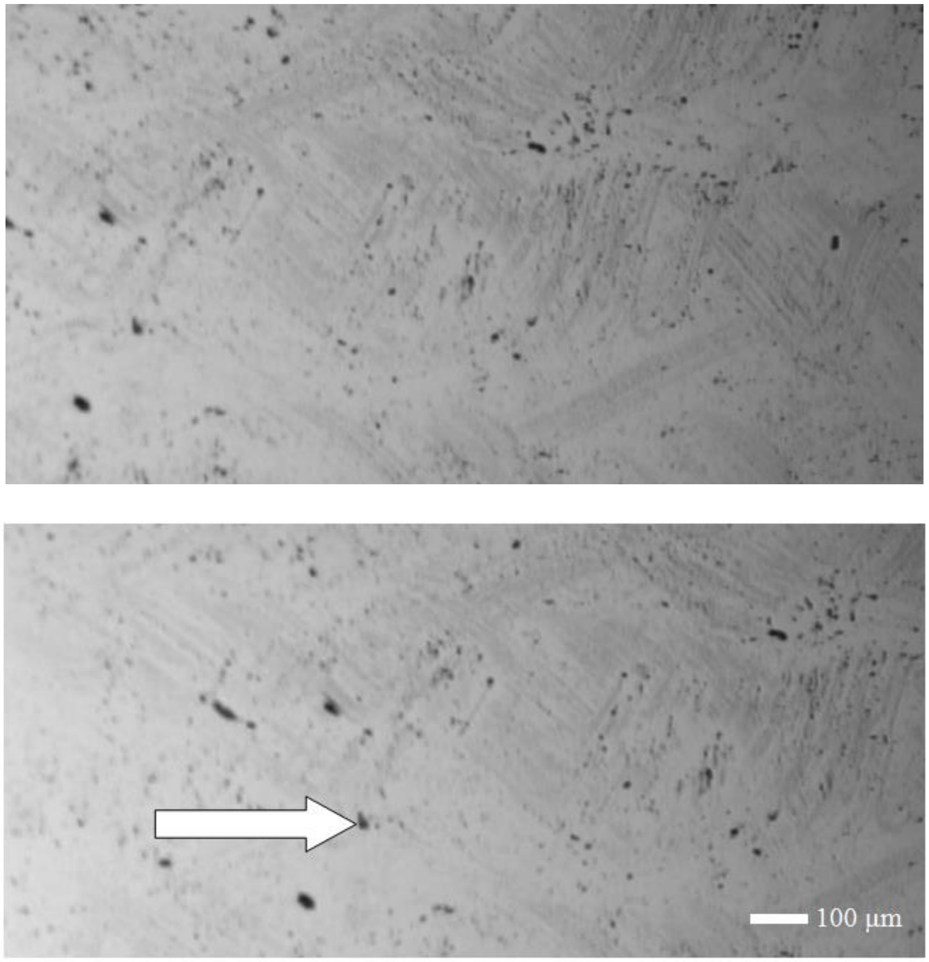

Figure 9: *Two images that represent the reference image (above) and an arbitrary image (below) of the silicon wafer surface. The arrow shows the distance traveled by the reference point, which can be measured using a software like ImageJ.*

The radius of curvature was determined to be 564 microns. The loads were averaged for each photo to give one load for the equation. The loads and contact radius could be used to calculate the K constant by applying a linear regression to the data of each SRT gel. From there, the Young's modulus could be calculated using the equation that relates K to Poisson's ratio and Young's modulus. The Poisson's ratio and Young's modulus of the indenter are known and the Poisson's ratio was assumed to be 0.5. That leaves K to be the only unknown variable in the equation. Table 1 and Figure 10 show the data necessary to calculate the modulus of one test of 6.25 wt% SRT to gel, which provided the highest modulus at 26.9 kPa.

Table 1: The data collected that is needed to calculate the Young's modulus for the 6.25 wt% SRT to gel test. An average of the mass measurements from the scale were taken, which were converted to force by multiplying by acceleration due to gravity (g_c). Then the distance determined from the ImageJ software was tabulated followed by the decompression value. From this value and the radius of curvature, a^(3/2) was calculated. The final columns are found by simple math operations and are used to make plots that will determine the Young's modulus of the gel.

Image Number	*M (g)*	*P (N)*	*Distance (pixels)*	*Delta (pixels)*	*Delta (mm)*	*a^3/2 (mm^3/4)*	*P/a^3/2 (N/mm^3/2)*	*(a^3/2)/R (mm2*
6 (reference)	0.000	0.00E+00	371	0	0	0	#DIV/0!	0
7	0.024	2.39E-04	368	3	0.006	0.014	0.017	0.025
8	0.141	1.38E-03	318	53	0.107	0.122	0.011	0.215
9	0.297	2.91E-03	279	92	0.185	0.184	0.016	0.326
10	0.435	4.27E-03	250	121	0.244	0.226	0.019	0.400
11	0.572	5.61E-03	217	154	0.310	0.271	0.021	0.480
12	0.789	7.73E-03	183	188	0.379	0.314	0.025	0.557
13	1.109	1.09E-02	142	229	0.461	0.364	0.030	0.646
14	1.621	1.59E-02	90	281	0.566	0.425	0.037	0.753
15	0.593	5.81E-03	161	210	0.423	0.341	0.017	0.605
16	0.256	2.51E-03	199	172	0.346	0.294	0.009	0.521
17	-0.112	-1.10E-03	251	120	0.242	0.224	-0.005	0.398
18	-0.435	-4.26E-03	307	64	0.129	0.140	-0.030	0.248
19	-0.540	-5.29E-03	357	14	0.028	0.045	-0.118	0.079
pixels/mm	*R (microns)*							
496	564							

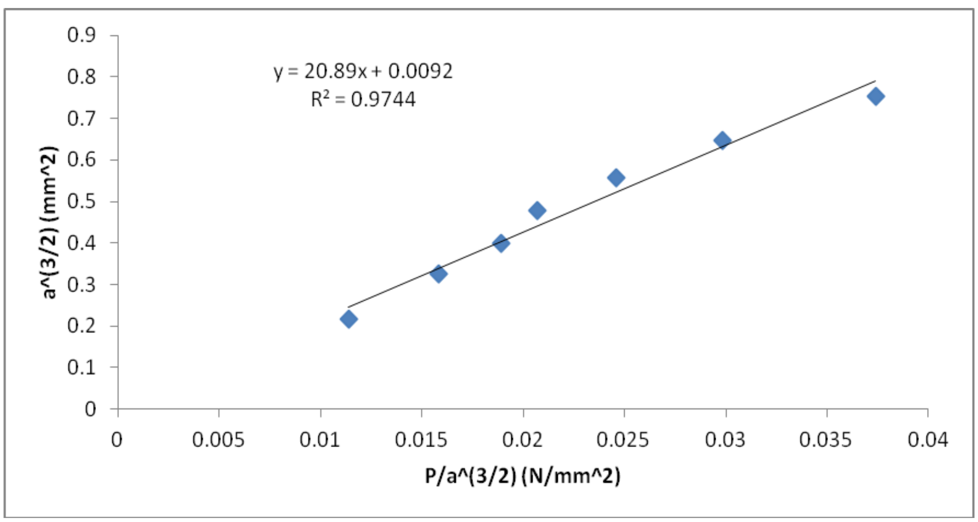

Figure 10: *A plot of the data for the 6.25 wt% SRT to gelatin hydrogel run. The slope of this graph is 1/K, and Young's modulus is derived from K. The equation with standard error is y = (20.89±1.51) * x + (0.0092±0.0365).*

Two runs of the samples were tested, with one having gels that were significantly drier (i.e., dry run) than the other run (i.e., hydrated run). Table 2 and Figure 11 show results of the dry run, while Table 3 and Figure 12 show results of the hydrated run. It is apparent that as the gel dries out, its modulus increases.

Table 2: Contact mechanics results of SRT showing peak at 6.25 wt% SRT in gel	
%SRT in solid	**Young's Modulus (kPa)**
0.00	4.313
1.10	6.047
2.17	12.985
3.23	17.023
5.26	13.407
6.25	26.927
7.22	13.361
8.16	4.005
9.09	16.561
10.00	8.889

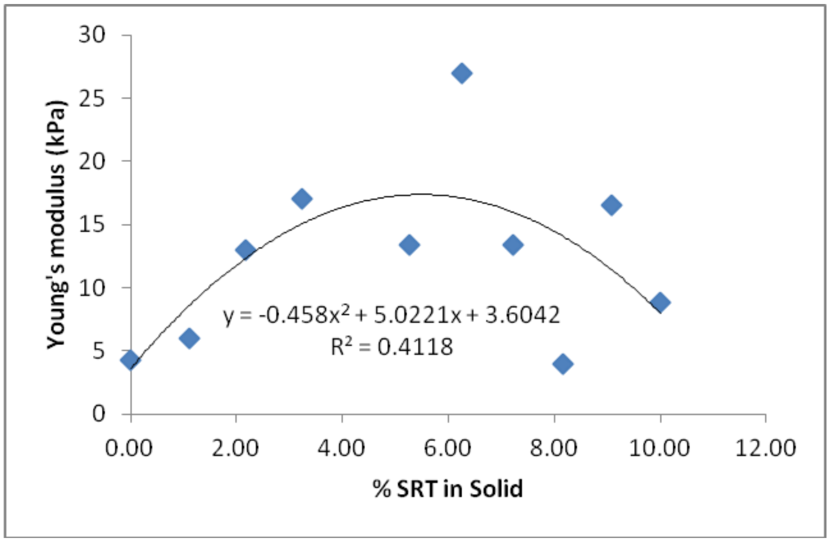

Figure 11: *The dry run plot of Young's Modulus vs. %SRT in solid data seen in Table 2. The constants of the equation were derived using a second order polynomial regression of the data and show no clear relationship. These figures are based on experiments using a 4.5 wt% hydrogel. The equation with standard error is* $y = (-0.458 \pm 0.221) * x^2 + (5.0221 \pm 2.2793) * x + (3.6042 \pm 4.7470)$.

Table 3: Contact mechanics results of SRT showing peak at 8.16 wt% SRT in gel	
%SRT in solid	**Young's Modulus (kPa)**
3.23	3.25
4.26	4.03
5.26	4.08
6.25	6.89
7.22	4.34
8.16	7.53
9.09	5.47
10.00	2.23

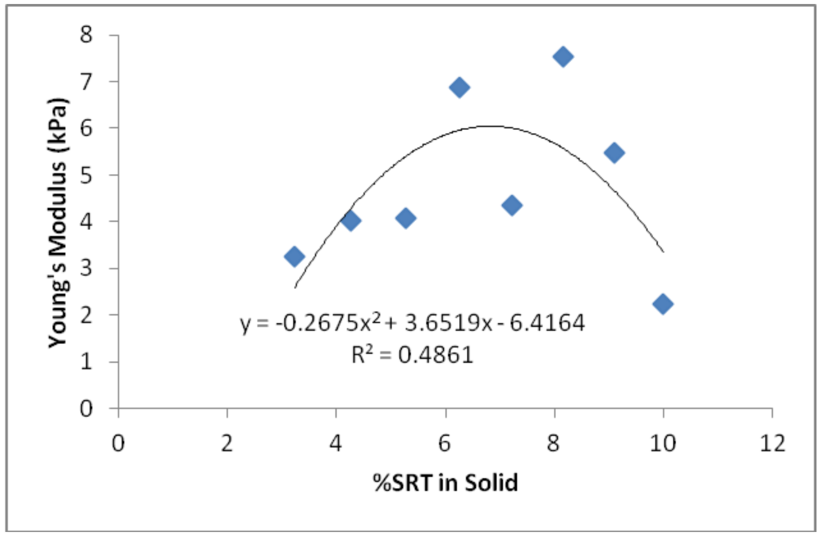

Figure 12: *The plot of Young's modulus vs. %SRT for the hydrated runs similar to the one seen in Figure 11. Again, dependable model is derived based on this data. The equation with standard error is* $y = (-0.2675 \pm 0.1256) * x^2 + (3.6519 \pm 1.6821) * x + (6.4164 \pm 5.1866)$.

These models show that the addition of SRT to gelatin affects the overall modulus in a parabolic manner. Further testing will have to be accomplished to determine whether or not this holds true.

Discussion and Analysis

SRT is capable of increasing the Young's modulus of gelatin. The highest Young's moduli were recorded at 6-8 wt% SRT to gelatin. It will take more work to figure out how much the Young's modulus can be increased and what SRT to gelatin ratios will bring about the best results.

The fact that SRT can strengthen gels makes SRT a viable candidate to be used as a filler or additive in the polymer processing industry to increase the strength or flexibility of polymers. Not only can it increase the strength of a polymer blend, but SRT behaves like a thermoplastic which makes it easier to process. Being a natural material, SRT also would bring a fairly clean synthesis process to an additive processing industry known for its mess and health hazards.

There was difficulty in creating uniform gels for testing mainly due to the loss of water in the hydrogel, and further research and thought into creating uniform gels would be valuable. Additionally, the dry tests were conducted on gels that were pulled out of the little vials they were created in, completely disrupting the uniform structure they had and adding a geometric variable to the equation. Despite efforts to keep the gels hydrated, they often dried significantly in a manner of hours. If an automated system to measure indentation location and load were available, it would be readily employed. Regardless, as was seen in other work[13], the Young's modulus of gelatin hydrogels was increased by adding SRT.

A final thought, could SRT be used to replace carbon black? Surely, this will not occur in the next 20 years. Carbon black is widely used and readily available and is well researched meaning that its utilization is understood well. SRT is still difficult to get in large amounts, even with recombinant SRT, and its research is still in early stages. If research continues and a facile manner of mass production is discovered, SRT may become an important commodity.

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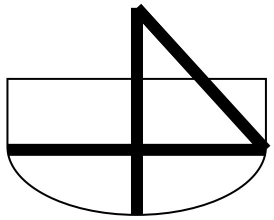
Appendices

Calculation of Radius of Curvature, R



Above is a depiction of the end of the capillary tube indenter. The bold horizontal line is the diameter of the capillary tube, d. The bold vertical line and the diagonal line depict the radius of curvature, R. The part of the bold vertical line below the bold horizontal line is called h. D and h can be measured using Yawcam and ImageJ software. This makes it possible to calculate R using the Pythagorean Theorem:

$$R^2 = (d/2)^2 + (R - h)^2$$

$$R^2 = (d/2)^2 + R^2 - 2Rh + h^2$$

$$R = \frac{(d/2)^2 + h^2}{2h}$$

If d = 398 microns and h = 79 microns:

$$R = \frac{(398\ microns/2)^2 + (79\ microns)^2}{2 * 79\ microns}$$

$$R = \frac{45842\ microns^2}{158\ microns}$$

$$R = 290\ microns$$

Calculating Contact Radius, a, from displacement, δ

$$a = \sqrt{R\delta}$$

If R = 564 microns and δ = 159 microns:

$$a = \sqrt{564\ microns * 159\ microns}$$

$a = \sqrt{89676\ microns^2}$

$a = 299\ microns$

Calculating K constant

K = 1/m when y = a^(3/2)/R and x = P/a^(3/2) and y = m*x

If a = 299 microns, R = 564 microns, and P = 1.67 x 10^{-3} N:

$$m = \frac{a^3}{R * P}$$

$$K = \frac{R * P}{a^3}$$

$$K = \frac{564\ microns * 0.00167\ N}{(299\ microns)^3}$$

$$K = \frac{564\ microns * 0.00167\ N}{(299\ microns)^3}$$

$$K = 3.52 * 10^{-8} N/microns^2$$

10^{12} microns^2 = 1 m^2, N/m^2 = Pa

$$K = 35235\ Pa = 35.235\ kPa$$

Calculating Young's Modulus, E

$$\frac{1}{K} = \frac{3}{4}(\frac{1 - \nu^2}{E})$$

If K = 35.235 kPa, and ν = 0.5:

$$\frac{1}{35.235\ kPa} = \frac{3}{4}(\frac{1 - 0.5^2}{E})$$

$$E = 19.820\ kPa$$