RESEARCH AND DEVELOPMENT OF SPIDER SILK FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

In January 2002, a 4-years project started at Ghent University concerning research on spider silk for biomedical applications. This project aims at the development of a textile fabric from spider silk. This textile fabric, produced from a biomaterial (proteins), has interesting medical applications. In order to be able to analyse the usefulness of spider silk to make a textile structure for biomedical applications, the influence of UV and steam sterilisation are investigated. For both sterilisation methods, more or less the same conclusions can be drawn: it results in a strong decrease of elasticity and toughness. The decrease in strength and stiffness is less clear, but is definitely not very high. Moreover it should be noticed that spider cocoon silk shows a high variability in properties within a cocoon and a high intraspecific variability between spiders.

1. Introduction

Like all orb-weaving spiders, *Araneus diadematus* makes to seven different kinds of fibres, each produced in a specialised gland and spun through a spinneret. Many labs are interested in the dragline silk as the strongest natural fibre known to man, a fibre that equals or even tops the qualities of high-tech synthetic fibres which are not biodegradable like protein-based silk. The extraordinary strength and elasticity can be explained by defining silk as a liquid crystal, combining amorphous regions inducing the elasticity and crystalline poly-Alanine containing ß-sheets causing the strength [1,2,3]. Sequencing cDNA's out of the glands after stimulating the spider to produce the silk of interest revealed different fibroin genes. These genes have a gland-specific expression-profile, so the coded proteins can polymerise to a fibre with the required mechanical properties [4]. Laboratory-scale expression of silk proteins is now feasible, in both bacteria and yeasts, and patents have been published about expressing silk genes in mammalian species and in the tobacco and potato plant [5]. This should make it possible to manufacture spider silk in large amounts.

Not all proteins coming from silk are known, especially the cocoon silk has not exposed many of his mysteries. The interest in the relation between the structure and the mechanical properties of cocoon silk and the idea that the egg sac fibre should have anti-microbial properties made it really interesting for us to compare this type of silk with dragline silk of the same spider species and to investigate its usefulness in biomedical applications.

2. Project summary

In January 2002 a project with a duration of 4 years will start at the department of Textiles of the Faculty of Applied Sciences of Ghent University concerning research on spider silk for biomedical applications. This research requires a multidisciplinary approach resulting into cooperation with the departments of Biology and Biochemistry of the Faculty of Sciences and with the Department of Physical Medicine and Orthopedic Surgery of the Faculty of Medicine, both from Ghent University as well.

This project investigates the feasibility of weaving spider silk into a textile structure for medical applications in surgery for elastic fixations and separations of tendons and ligaments. Spider tissue will be woven on lab-scale in order to determine the chemical and physical properties. Subcutaneous implantations in rats will be performed to obtain a first evaluation of this biodegradable textile.

3. Preliminary experiments

In a first phase, the effect of sterilisation (UV-light and autoclavage) on spider cocoons is investigated. At the start of the project, only egg cocoons of the spider species *Araneus diadematus* could be collected. Since this spider species died after making a cocoon with eggs, in January no living *A. diadematus* spiders could be found for forced silking of dragline threads.

It is our aim to breed different spider species in the laboratory in order to compare different spider silks of different spider species allowing to correlate different characteristics such as spider species, spider weight and the properties of different silks.

4. Materials and methods

4.1. *Collection of the material*

The egg cocoon silk for the tests in this work was taken from egg cocoons made by *Araneus diadematus* spiders found in free nature.

As a first step in the preparation of the samples, the eggs were removed from the cocoons. After this, the threads were drawn bit by bit from the remaining silk-cluster under a magnifying glass and hung in the holders of a single fibre tensile tester to be tested.

4.2. *Stress-strain measurements*

The stress-strain measurements have been performed with an automatic single fibre tester, the FAVIMAT-ROBOT. This instrument measures simultaneously the fineness (dtex) that allows determining the tenacity (cN/tex), an often used parameter for evaluating the strength of fibres. Because of the high variability of fineness for spider silk, relative values are required in order to allow comparisons.

The measurement of the linear density is based on the vibration method at constant fibre tension and fibre length and variable stimulating frequency.

Tensile tests with the FAVIMAT are carried out according to the constant rate of extension principal.

All tests were done at a controlled environmental condition of 20 ± 2 °C and 65 ± 2 rH.

4.3. *The Xenon-Test*

The Xenon test or Xenon arc test is a test that is used to measure the light- and weathering fastness of materials. The Xenon test is especially used when good agreement with outdoor testing is the major goal. In this test the natural weathering process is accelerated by exposing the sample under prescribed conditions to artificial sunlight (xenon arc light) for a certain time period.

The Xenon test enables the evaluation of the effect of UV-sterilisation on spider silk.

4.4. *Steam sterilisation*

Although many microorganisms are killed by comparatively low temperatures, others (eg bacterial spores) are much more resistant. Steam sterilisation is normally the most efficient and convenient method for equipment, media and contaminated materials. A holding time and temperature policy such as 18 minutes at 121°C (1.1 kg/cm2 or 15 psi) should be chosen. This process is also called autoclavage.

5. Results and discussion

5.1. *Effect of UV-light on stress-strain measurements (Table 1)*

The effect of UV-light is investigated by using the Xenon-test on 5 egg cocoons of the spider species *Araneus diadematus.* For each egg cocoon 50 repetitions were made, however not all tests succeeded. The stress-strain measurements were determined before and after the Xenon-test.

Testing parameters for the tensile test were: a gauge length of 20 mm, a test speed of 20 mm/min and a pretension of 0.50 cN/tex. For the linear density test, a test speed of 5 mm/min and a pretension of 0.80 cN/tex were used.

A t-test is performed on the results to evaluate the effect on the mechanical properties.

	Cocoon 1		**Cocoon 2**		**Cocoon 3**		**Cocoon 4**		**Cocoon 5**		**Mean**	
	Bxen	**Axen**	**Bxen**	**Axen**	**Bxen**	**Axen**	**Bxen**	**Axen**	**Bxen**	**Axen**	**Bxen**	**Axen**
ε - mean	25.77	**15.37**	37.51	**14.00**	28.07	**7.78**	29.37	**16.51**	22.72	**6.31**	28.93	**12.03**
ε - CV	50.58	71.12	56.82	82.38	54.17	45.08	63.44	65.68	60.14	86.64	60.74	82.58
F - mean	1.56	**1.36**	2.25	2.08	1.67	**1.35**	1.82	**2.00**	1.69	**1.31**	1.81	**1.63**
F - CV	23.45	35.54	27.84	28.94	27.49	24.94	36.77	20.46	20.88	29.11	31.73	34.87
W - mean	0.80	**0.48**	1.70	**0.73**	0.97	**0.21**	1.13	**0.68**	0.74	**0.27**	1.08	**0.50**
W - CV	50.00	62.50	51.76	71.23	57.73	47.62	63.72	69.12	62.16	51.85	67.05	84.04
f - mean	2.22	**1.86**	2.19	**1.86**	2.09	**1.82**	2.05	2.05	1.73	**1.38**	2.05	**1.80**
f - CV	17.32	32.35	22.91	27.73	19.43	18.62	27.77	17.36	22.32	27.93	23.91	27.76
E - mean	60.18	59.05	55.00	57.68	56.15	**61.47**	54.48	**61.53**	49.09	50.00	54.89	**58.12**
E - CV	7.94	8.94	10.23	9.16	12.28	11.29	11.42	7.32	10.18	21.69	13.04	13.73
dtex - mean	0.71	0.75	1.03	**1.11**	0.80	**0.74**	0.89	**0.97**	0.98	0.97	0.89	0.91
dtex - CV	19.01	18.87	14.73	4.84	17.59	16.33	24.67	8.10	13.99	18.41	21.29	19.62

Table 1: Tensile properties and linear density of the 5 egg cocoons before (Bxen) and after (Axen) the Xenon test with ε elongation at break (%) , F force to break (cN), W work to rupture (cN*cm), f tenacity (cN/dtex), E modulus at 2% (cN/dtex) (bold values indicate a significant difference $p < 0.05$)

- Elongation at break ε (%): A general trend for all the cocoons is the very strong significant decrease (40-70 %) in the elongation at break. So it is definitely permitted to conclude that cocoon silk becomes less elastic due to the exposure to UV-light. In comparison to dragline silk, it can be concluded that the elongation is similar (28 % for dragline silk [6]).

- Force to break F (cN): On the other hand, for force to break it is difficult to make a conclusion. For most cocoons (except cocoon 4) there is a significant decrease (10-20%). For cocoon 4, the force to break is 10 % higher after the Xenon test.

- Work to rupture W (cN*cm): For the influence on work to rupture it can be concluded that the exposure to UV-light results in a significant decrease (40-80%) of work to rupture, in other words, less energy is required to break the spider silk. It should be remarked that the difference is very variable between the different cocoons.

- Tenacity f (cN/dtex): The tenacity of fibres takes into account the linear density (which is also measured by the FAVIMAT instrument). For this parameter, for all cocoons, except cocoon 4, the tenacity decreases significantly (10-20 %). For cocoon 4, there is no difference measured for and after the Xenon-test. The absolute tenacity amounts to 1.5-2 cN/dtex that is considerably lower (4 times) than the value for dragline silk (± 8 cN/dtex [6]).

- Young's Modulus at strain 0.02 E (cN/dtex): For the effect of UV-light on the modulus, no clear conclusion can be made. For the cocoons 1, 2 and 5, no significant difference is observed whereas for the cocoons 3 and 4, there is a significant increase (10-15%). So, it appears that the stiffness of spider silk will rather increase than decrease when exposed to UV-light. The absolute value of the modulus is comparable to data found in literature for dragline silk (6,90 GPa or 53 cN/dtex [6]).

- Linear density (dtex): Since the FAVIMAT instrument also allows measuring the fineness in dtex, this parameter is evaluated. For the linear density, it appears that UV-light does not have much influence, which could be expected. The differences observed in terms of percentage amount to less than 9%. As can be seen, the absolute fineness of the cocoon silk lies in the neighbourhood of 0,9 dtex. Cocoon silk has the same fineness as dragline silk (3,0 µm or 0,9 dtex [7]).

5.2. Effect of steam sterilisation on stress-strain measurements (Table 2)

The stress-strain measurements were determined before and after steam sterilisation for 5 egg cocoons of the spider species *Araneus diadematus*. For each egg cocoon 50 repetitions were made, however, not all tests succeeded.

Testing parameters for the tensile test were: a gauge length of 10 mm, a test speed of 5 mm/min and a pretension of 0.50 cN/tex. For the linear density test, a test speed of 1 mm/min and a pretension of 0.25 cN/tex were used.

A t-test is performed on the results to evaluate the influence on the mechanical properties.

	Cocoon 1		**Cocoon 2**		**Cocoon 3**		**Cocoon 4**		**Cocoon 5**		**Mean**	
	Bclav	**Aclav**	**Bclav**	**Aclav**	**Bclav**	**Aclav**	**Bclav**	**Aclav**	**Bclav**	**Aclav**	**Bclav**	**Aclav**
ε - mean	48.54	**31.74**	43.92	**31.78**	42.21	**29.27**	42.68	**26.15**	42.43	**27.91**	43.98	**29.32**
ε - CV	35.92	33.82	31.88	40.97	36.12	40.93	43.05	45.22	39.20	40.84	45.04	40.63
F - mean	1.99	1.94	1.88	**1.77**	1.94	**2.37**	2.06	2.00	1.55	1.62	1.89	1.94
F - CV	14.30	19.59	19.60	8.02	12.45	7.57	13.07	10.58	23.49	13.10	18.41	17.99
W - mean	0.79	**0.53**	0.66	**0.48**	0.69	0.60	0.70	**0.42**	0.54	**0.38**	0.68	**0.48**
W - CV	43.20	38.22	44.38	43.42	41.36	41.71	49.15	50.47	50.19	46.35	54.85	46.65
f - mean	2.38	2.30	2.62	**2.28**	2.13	**2.35**	2.64	**2.45**	2.12	2.20	2.38	2.32
f - CV	17.16	19.74	17.90	20.09	14.84	15.65	18.68	18.85	21.16	11.92	20.61	17.78
E - mean		43.39	49.14	47.59	47.26	50.67	48.56	**43.99**	43.86	42.72	47.17	45.64
E - CV		18.47	16.80	21.06	18.57	15.83	15.14	22.96	22.01	16.64	18.84	20.08
dtex - mean	0.87	0.87	0.73	0.75	0.93	**1.03**	0.79	0.83	0.76	0.74	0.82	0.84
dtex - CV	24.23	24.01	21.45	17.66	17.33	11.57	13.29	12.53	37.18	14.35	24.50	20.44

Table 2: Tensile properties and linear density of the 5 egg cocoons before (Bclav) and after (Aclav) autoclavage with ε elongation at break (%) , F force to break (cN), W work to rupture (cN*cm), f tenacity (cN/dtex), E modulus at 2% (cN/dtex) (bold values indicate a significant difference $p < 0.05$)

- Elongation at break ε (%): As for the Xenon-test, it is obvious that the elongation at break significantly decreases (25-40%) after steam sterilisation. Also in this case, it is permitted to say that spider silk becomes less elastic after steam sterilisation.
- Force to break F (cN): No conclusions can be drawn from the results. For cocoon 2, a significant decrease is perceived while for cocoon 3 a significant increase is observed. However, a t-test on all values reveals no statistical difference (± 3%).
- Work to rupture W (cN*cm): It can be concluded that the energy to break for spider silk is significantly lower (25-40%) after autoclavage. So, spider silk becomes less tough by autoclavage.
- Tenacity f (cN/dtex): As for force to break, it is difficult to make conclusion. For cocoons 3 and 5, the tenacity seems to increase, what is not expected. For cocoon 4 and 5, a significant decrease (± 10%) in stiffness is observed. It can be concluded that the tenacity will not decrease a lot after steam sterilisation. A t-test on all values shows no statistical difference (± 3%).
- Young's Modulus at strain 0.02 E (cN/dtex): Only for cocoon 3 the value increases, the difference is not significant. So, it is permitted to conclude that the modulus will decrease after autoclavage, however the degree is small (< 12%). Taking all values together results in a small (not significant) decrease of 3%.
- Linear density (dtex): it appears that the linear density is not affected. Only for cocoon 3 a significant increase (±11%) is noticed.

5.3. General discussion

First of all, it should be remarked that care is to be taken when interpreting statistical results because of the high variability in the results, which is typical for natural fibres.

Moreover, spider silk is a special extremely fine fibre, which gives sometimes problems during the stress-strain testing.

It can be noticed that the values determined in the first series of measurements are different in absolute value than those of the second series of measurements. First of all, it should be remarked that the cocoons used for the Xenon test and the sterilisation test were different ones. Another reason for the difference is probably the different parameters used on the FAVIMAT-instrument (different test speed and gauge length). The influence of each of these parameters will be investigated later on.

6. Conclusion

It should be remarked that the high variability in mechanical properties for dragline silk is also observed in the properties of egg cocoons [6]. This variability will be further investigated for other spider species.

In comparison to dragline silk [6], it can be concluded that the elongation and modulus are similar while the tenacity is considerably lower for cocoon silk.

It can be expected that UV sterilisation will result in a less elastic, a less strong, and probably a little more stiff spider silk. The work to break of the UV sterilised spider silk is significantly lower. As could be expected, UV-light has no influence on fineness.

It is permitted to conclude that steam sterilisation strongly lowers the elasticity and the toughness of spider silk. In respect to strength and stiffness that can be relevant for biomedical applications, no clear conclusion can be made, however it could be expected that only a small decrease (< 12%) will occur.

It is surprising that UV-light appears to have a more pronounced influence than steam sterilisation. This will be further investigated.

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8. References

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