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# $SiO_2$ Entrapment of animal cells

## Part I *Mechanical features of sol-gel $SiO_2$ coatings*

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Natural silk fibers show valuable changes in elastic modulus, $E$, and failure stress, $\sigma_f$, upon treatment with an air flux of gaseous Si-alkoxides bearing a thin sol-gel layer of $SiO_2$ on the fiber surface. These mechanical features are studied here as a function of the composition of gaseous flux and reaction time. Owing to the different behavior between original and treated fibers submitted to loading-unloading cycles, the maximum increase in $E$ (about 50%) and $\sigma_f$ (about 30%) are discussed in terms of intermolecular surface interactions of -O-Si-O- bridging groups and protein macromolecules. As an extension of this system, composed of a thin sol-gel $SiO_2$ layer (0.05–0.1 $\mu$m) on protein surfaces, the mechanical improvement of collagen + cell deposits upon deposition of sol-gel $SiO_2$ is suggested.

## 1. Introduction

Some of the diverse arrays of sol-gel $SiO_2$ span the incorporation of functional organic molecules and bioactive species, including dyes [1], enzymes [2–4] and even cells [5–8]. As for living cell entrapment, two independent approaches have been proposed: (1) integration of cells even during $SiO_2$ sol-gel processing [5, 6] adjusted to fulfil restrictions concerning handling of living systems, and (2) build-up of a sol-gel $SiO_2$ coating, directly on the cell surface [7, 8] by exploitation of basic chemical reactivity of the silicon-alkoxide precursors. This second method, called Biosil [9, 10], is illustrated in Scheme 1.

Living cells are left to adhere to fibers of scaffolding materials with selected mechanical properties and textured to avoid mass-transfer limitations, were they are invested by an air flux of silicon-alkoxide precursors. The silica layer originates from reaction of surface hydroxides and alkoxides; sol-gel thickness may be controlled by the time of treatment and by the concentration of alkoxide in the air flux, which removes excess reagent and volatile toxic by-products.

In addition to specific applications in vegetable cell production of secondary metabolites [7] and pancreatic islet entrapment [8] this method appears to be an alternative or valuable addition to reported techniques in the design of hybrid bio-artificial liver [11]. A large variety of bio-reactors, containing viable hepatocytes has been proposed [12]. Bio-reactors design based on multi-plated matrix-overlaid hepatocytes, provides an attractive organo-typical approach [13], mimiking the situation *in vivo*. Limitations of these systems are poor matrix resistance to shear stress exerted by patient plasma flowing through the system and the lack of immuno-isolation. These shortcomings could be circumvented by improvement of incapsulation technology. Preliminary results obtained with the Biosil method indicate that deposition of a 0.1–0.2 $\mu$m silica layer on the surface of collagen-entrapped hepatocytes can be achieved with preservation of cell viability and functionality. Collectively, these features are quite promising for the purpose at hand: indeed, experiments have been carried out with the aim of ascertaining whether the method of Scheme 1 may be exploited for bio-artificial liver, neglecting the definition of specific features implied in the extension of the sol-gel technique to complex biological systems.

Current interest in this subject spurred us to organize explorative work in order to investigate: (i) the nature of the composite sol-gel $SiO_2$ + collagen and inherent mechanical features, (ii) the barrier effect vs. macromolecule transport across the collagen + $SiO_2$ composite, and (iii) the metabolism of entrapped hepatocytes for typical hepatic functions.

In this first work we report the results regarding point (i).

Collagen layers, promoting the mosaic-like structure of hepatocytes, features a thickness of few hundreds of microns; they are gels composed of a protein network immobilizing the solvent, the solid mass being about 0.1% in volume. Owing to this feature, the chemical, physical and mechanical characterization of

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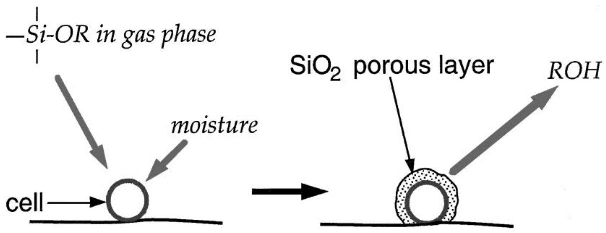

*Scheme 1*

sol-gel $SiO_2+$ collagen films can not be performed by ordinary methods. Consequently, representative samples, i.e., silk fibers were used, so that collected data could furnish evidence for qualification of the $SiO_2+$ collagen composite.

## 2. Experimental

### 2.1. Materials

$Si(OEt)_4$ (TEOS) and $CH_3SiH(OEt)_2$ (DEMS) were purchased from Petrarch System and used as received. Other chemicals were commercially available analytical reagent-grade products used as received.

Glassy carbon sheets (18 mm × 18 mm × 1 mm), used as supports for sol-gel $SiO_2+$ collagen composites, were purchased from Carbone Lorraine (Paris, France).

Silk fibers were pulled from an authentic cocoon of Indian silkworm. After washing in distilled water at 80 °C for 1 h, they were mounted in a frame, ranked and stretched between tips, a magnifying glass being used to fit a dozen single fibers ($\approx$9 cm in length) in each frame. Some fibers were observed by a Scanning Electron Microscope (Cambridge Stereoscan, Cambridge, UK) to have a diameter of $14 \pm 1$ $\mu$m.

Collagen was purchased from Sigma (Type 1 collagen) and used in a 1 mg/cm$^3$ water solution.

### 2.2. Preparation of samples

According to the Biosil method [7, 8], a gaseous flux of silicon alkoxides was obtained by saturation of an air flow by bubbling into a solution of TEOS + DEMS, kept at 80 °C; molar percentages of these compounds in solution were 80 + 20, 70 + 30 and 60 + 40. A gaseous flux of 400 cm$^3$/min was used in all cases.

Metal frames, each holding a dozen silk fibers, were introduced in a 1800 cm$^3$ glass cylindrical reactor equipped with a main entrance, exit and inspection port for temperature control. Samples were invested for 15 min by 500 cm$^3$/min air flux saturated with $H_2O$ at 40 °C; after 10 min airing with nitrogen (400 cm$^3$/min), fibers were reacted with the gaseous alkoxide flux at 35 °C, extracted from the reactor and put aside in air for some days before mechanical testing. Three TEOS + DEMS compositions were used; in the case of the 80 + 20 mixture, reaction times were 5, 10 and 15 min; for 70 + 30 and 60 + 40 compositions, a 10 min treatment was adopted.

Polystyrene cups, 3 cm in diameter and 1 cm in height, were coated with a 630 $\mu$m (calculated thickness) layer of collagen, using the procedure reported in the literature [14, 15]. After careful elimination of the excess liquid, cups were treated as well as the silk fibers, i.e., 10 min airing and reaction with the 80 + 20 alkoxide flux at 35 °C, for 2, 4, 6, 9, 12 and 18 min. The cups were kept under vacuum under $10^{-3}$ torr for one night; Si elemental analysis of the dry residue was determined by Neutron (Modena, Italy) using an ICP apparatus (Faison's Instruments).

Glassy carbon sheets were treated at the perimeter rim with sticky tape, providing a 9 mm high edge; the collagen solution was poured onto the carbon surface and homogeneously arranged to obtain a 520 $\mu$m (calculated thickness) layer of collagen. Obtained samples were reacted as described for polycarbonate cups for 10 min and put aside in air.

### 2.3. Mechanical tests

Elastic modulus, failure stress and maximum elongation of original and treated single fibers were measured by tensile tests. Fiber edges were bonded to two small steel rings with an organic adhesive. This allowed the connection of the fiber to the testing machine by two steel hooks. Tensile tests were performed at $25 \pm 2$ °C using a universal mechanical testing machine (Instron, model 4502, Canton, MA, USA) equipped with a load cell of 1 N maximum load. Tests were conducted with a cross head speed of 10 mm/min. About ten measurements were performed for each condition.

## 3. Results

Silk fibers are natural protein products displaying a variety of features depending on the feeding and selection of the silkworm: we used silk fibers drawn from a single cocoon in order to reduce the scattering of physical and chemical parameters. Treatment with hot water removes contaminants on the fiber surface; in some cases, twin fibers are considered owing to the difficulty of separating individual fibers with length suitable for mechanical testing.

Fiber surface treatment with gaseous TEOS + DEMS involves different times and compositions: obtained samples are reported in Table I.

TABLE I Sample labelling and preparation

| Sample | | Composition of alkoxide solution[a] | Exposure time (min) |
|---|---|---|---|
| Silk fiber | $S_0$ | Untreated | — |
| Silk fiber | $S_{80}^{5}$ | 80 + 20 | 5 |
| Silk fiber | $S_{80}^{10}$ | 80 + 20 | 10 |
| Silk fiber | $S_{80}^{15}$ | 80 + 20 | 15 |
| Silk fiber | $S_{70}^{5}$ | 70 + 30 | 5 |
| Silk fiber | $S_{60}^{5}$ | 60 + 40 | 5 |
| Collagen on polystyrene cup | $C_{80}$[b] | 80 + 20 | 2, 4, 6, 9, 12, 18 |
| Collagen on glassy carbon | $G_{80}$[c] | 80 + 20 | 10 |

[a] Molar ratio of TEOS + DEMS.
[b] Thickness of collagen layer = 630 $\mu$m.
[c] Thickness of collagen layer = 520 $\mu$m.

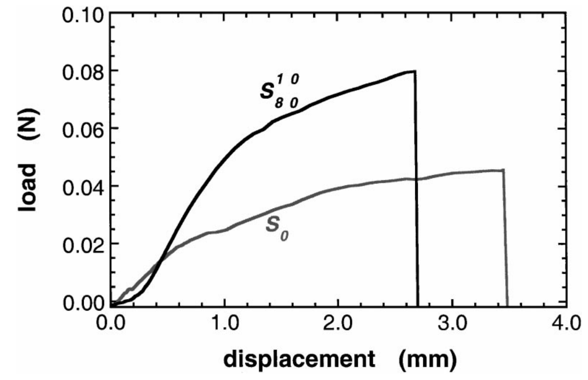

*Figure 1* Typical load-displacement diagram for original and $S^{10}_{80}$ samples.

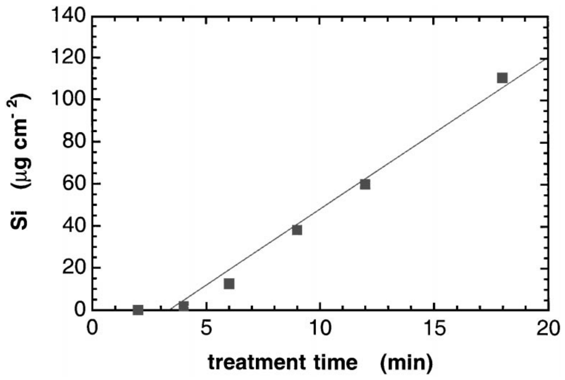

*Figure 2* Si elemental analysis according to exposure time in $C_{80}$ samples.

A typical load-displacement diagram of fibers is shown in Fig. 1. The behavior is expected of polymeric materials with an initial elastic regime followed by a plastic deformation up to failure point. Elastic modulus, $E$, was evaluated from maximum slope, $m$, of the load-displacement diagram by the following relation:

$$E = mL_0/A \tag{1}$$

where $L_0$ is the length and $A$ the cross-section of the fiber. Equation 1 may be used rigorously as the compliance of the testing machine and of the used fixtures was of some orders of magnitude smaller than the compliance of the fibers.

Failure stress, $\sigma_f$, was calculated on the basis of maximum load, $P_{max}$, as:

$$\sigma_f = P_{max}/A \tag{2}$$

Maximum elongation, $\Delta l_\%$, was obtained by the equation:

$$\Delta l_\% = \Delta l_f/L_0 \tag{3}$$

where $\Delta l_f$ is elongation at failure.

Elastic modulus, failure stress and maximum elongation results are reported in Table II.

The experimental conditions selected for the gas phase reaction of alkoxides with the fiber surface were maintained for preparation of gel-like $SiO_2$ on planar collagen deposits. For the 80 + 20 (TEOS + DEMS) composition at various raction times, Si elemental analysis of the $SiO_2$ + collagen dry solid indicates the linear dependence of Si content on treatment time (Fig. 2), corresponding to 4.6 $\mu$g cm$^{-2}$ min$^{-1}$. The effectiveness of this relationship starts after ≈4 min treatment, the presence of this lag time being associated with reactor volume (1800 cm$^3$) vs. gaseous flux (400 cm$^3$/min). Taking into account a sol-gel $SiO_2$ density of 1 g/cm$^3$, the thickness of deposited silica is calculated not to exceed 0.01 $\mu$m per minute of effective treatment, i.e., starting with 4 min lag time.

TABLE II Mechanical tests results[a]

| | $E$ (MPa) | $\sigma_f$ (MPa) | $\Delta l_\%$ (%) |
|---|---|---|---|
| $S_0$ | 6112 ± 1909 | 233 ± 68 | 8.6 ± 3.6 |
| $S^{10b}_{80}$ | 10286 ± 1436 | 289 ± 47 | 8.3 ± 3.2 |
| $S^{5}_{70}$ | 7153 ± 1293 | 283 ± 67 | 11.1 ± 6.1 |
| $S^{5}_{60}$ | 9490 ± 1577 | 299 ± 34 | 9.9 ± 2.7 |
| $S^{5}_{80}$ | 6334 ± 2313 | 142 ± 5 | 7.8 ± 5.5 |
| $S^{15}_{80}$ | 7239 ± 1692 | 207 ± 9 | 8.1 ± 2.6 |

[a] Standard deviation from 9 ÷ 12 measurements.

[b] Standard deviation from 19 measurements.

SEM observations of $SiO_2$ + collagen deposits on a glassy carbon surface revealed that the collagen layer had collapsed, owing to water evaporation in vacuum. Elemental analysis of this residual solid by EDXS indicated the presence of Si.

## 4. Discussion

Natural silk is a protein system which requires preliminary working-up for the preparation of commercial hanks. The final product has an elastic modulus of about 10 GPa and strength around 300 MPa. Two proteins constitute the main components of this material, structurally organized to afford a non-crystallized solid. After reaction with gaseous alkoxides, the protein mass is preserved and the sol-gel $SiO_2$ mass can not be directly measured.

Upon extension of the data in Fig. 2 to $SiO_2$ deposition on silk fibers, the silica layer thicknesses were 0.01, 0.06 and 0.11 $\mu$m for 5, 10 and 15 min treatment, respectively (80 + 20 composition). This negligible supply to the fiber mass prevents discussion of the data in of Table II in terms of mere additional contribution of the $SiO_2$ sol-gel layer to the original $E$ and $\sigma_f$ values of untreated fibers.

A detailed study of the load-displacement diagrams was performed by analyzing elastic recovery at various loads, some samples being subjected to loading and unloading cycles before failure. Fibers show elastic behaviour for stresses lower than ≈100 MPa (Fig. 3); plastic deformation is observed for higher stress levels. In this plastic regime, an appreciable difference between original and treated fibers is evident; as shown in Fig. 3, original samples show similar elastic modulus during successive loadings, even for loads higher than the yielding stress. Conversely, as increasing loads were applied to treated fibers, initial stiffness decreased during the loading and unloading cycles.

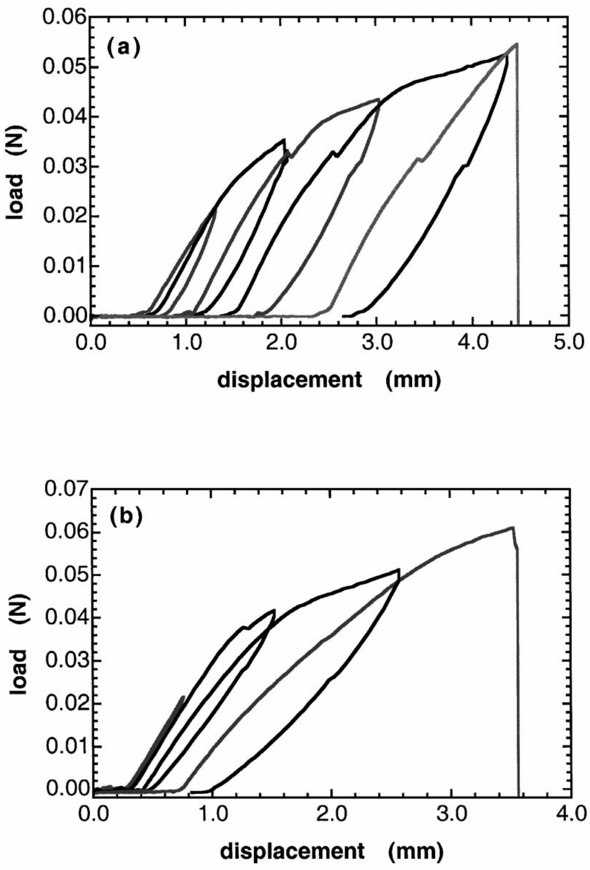

*Figure 3* Load-displacement diagrams illustrating loading and unloading cycles for (a) original and (b) $S_{80}^{10}$ samples.

This phenomenon suggests that gaseous Si-alkoxide treatment modifies the intrinsic mechanical features of silk fibers, investing the microstructure and intermolecular bonding of proteins. Accordingly, the considerable increases in $E$ and $\sigma_f$ (Table II) may be discussed by focusing on the occurrence of chemical bonds by reactive alkoxides and silk proteins. The varying presence of terminal OH groups in protein structures and the presence of water molecules engaged by hydrogen bonds or sorbed on their surface, favour chemical interactions with gaseous Si-alkoxides. Although the variety of potential reactive sites hinders precise definition of chemical reactions, the occurrence of bridging -O-Si-O- moieties may result in a considerable increase in elastic modulus. Loading and unloading cycles in the plastic regime remove these -O-Si-O- blocks, accounting for the observed decrease in $E$ after the yielding point. According to this interpretation, involving a structural modification operated by -O-Si-O- moieties on protein macromolecules of silk fibers, the similarity between original and treated samples as regards maximum elongation (Table I) appears justified, since maximum elongation corresponds to the total failure of intermolecular bonds.

The data of Table II suggest that composition of gaseous flux and reaction times affect mechanical properties, although the scatter prevents definite comparison of results. In general, the tetrafunctionality of TEOS should favour the occurrence of intermolecular bonding for gaseous compositions rich in this precursor.

In conclusion, expectations in terms of mechanical improvement induced by the deposition of a very thin layer of sol-gel $SiO_2$ on a natural protein surface results in a maximum increase of about 50% in the original elastic modulus. In the design of bio-artificial organs, our data indicate that stress limits which interfere with cell organization may be significantly extended by interaction of the protein collagen layer with Si-alkoxides.

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