Nicolaus Copernicus University Instituto de Engenharia Biomédica University of Porto

Scientific Report for Short Term Scientific Mission

COST Action MP1301

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Investigation on molecular interaction between Collagen and Silk Fibroin in order to prepare an optimum biocomposite for bone regeneration

By

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Purpose of STSM

The main purpose of this mission was to analyse the miscibility of the Collagen/Silk Fibroin blends as templates for bone mineralization. Blending of these two biopolymers can compensate the weakness of each ones and result in a new biocomposite with better and newer properties as well as better economical aspects. Proper blending depends on the degree of compatibility or miscibility of the two biopolymers at a molecular level which is identified by specific interactions such as dipole forces, hydrogen bonding and charge transfer complexes between the polymer segments.

Among several techniques that have been introduced for analysing the miscibility, viscometry and XRD techniques have been chosen for this research due to the low cost and capacity to obtain quick results of these techniques. Viscometry is a simple technique to observe the interactions between polymers in blends. On the other hand, according to the literature, it is presumed that X-Ray can give information on the crystal structure of silk fibroin and its conformation as silk I or silk II through blending with collagen.

Reviewingthe available bibliography showed that no work has been done on the miscibility of collagen and silk fibroin blends.

The group of Professor Alina Sionskowska at Nicolaus Copernicus University (NKU) in Tórun, Poland, has good experience on the blending of different biomaterials and analysing their molecular interactions. So, with the help of this group, we investigated the miscibility of Collagen and regenerated Silk fibroin through Viscometry and XRD techniques.

Description of the work carried out during the STSM

Collagen solution was prepared by dissolving collagen bovine type I in 0.5 molar of acetic acid and stirred under high speed Torrax at 4 °C for 2-3 hours. Afterwards, the solution was casted as film in polystyrene petri dishes at room temperature for XRD measurement.

Silk fibroin solution was prepared by dissolving silk fibroin (made from cocoons of the silkworm *Bombyx mori*) in a ternary solvent of CaCl2:CH3CH2OH:H2O solution. The final solution was dialyzed against water and subsequently casted in petri dishes to form films for further XRD analysis.

The collagen/SF blends were prepared by mixing different ratios with the attempt to prevent collagen denaturation. Afterwards, the blending solutions were cast in petri dishes to form films for XRD measurement. The XRD was carried out at 30 kV and 20 mA, a speed rate of 0.5 °/min, and in the range of 2θ from 0-50.

The viscosity behaviour of aqueous mixtures formed by collagen and silk fibroin was investigated at 25 °C by Ubbelohde capillary viscometer. The intrinsic viscosity and the viscosity interaction parameter of each polymer solution as well as the mixtureswere determined and served for estimation of the miscibility of different polymer mixtures by classical dilutionmethod.

Description of the main results

In this research, the pure polymers, the mixtures and a simple electrolyte (as the solvent) were investigated for analysing viscosity. Miscibility of collagen and silk fibroin blends before dialysis has been studied by viscometry in dilute solution with NaCl (0.1 M) as the solvent, at 25 °C. By the method of classical dilution, the miscibility parameter (Δb_m) as well as relative and reduced viscositieshave been calculated both theoretically (by methods of Krigbaum and Wall [Ref], and Garcia [Ref]), and experimentally and plotted against solutions concentrations. The difference between the experimental and theoretical (ideal) values identifies if the system is miscible. In fact, Krigbaum et al. suggested that the miscibility of the polymer blends is based on interaction parameters. According to their theory, the two polymers are miscible if $\Delta b_m \geq 0$and immiscible when $\Delta b_m \leq 0$.

The data obtained from the theoretical and experimental values suggest that the miscibility parameter is positive for all the mixtures without dilution. But with adding the dilution solvent, this parameter is negative. The results indicate that the pure mixtures without dilution have good miscibility because opposite charges in the whole mixtures are in balance, while adding the solvent disturbs

the stability of charges which is unfavourable and causes immiscibility. Hence, it can be concluded that the polymers molecules in the prepared mixtures are electrostatically bond to each other and makes a uniform and stable solution which is proper for subsequent researches.

Moreover, the plot of reduced viscosity versus the concentration for pure collagen, pure silk fibroin and their blends illustrates thatsilk fibroin has the lowest viscosity due to the domination of silk I conformation in the solutions. Collagen which has higher viscosity than silk fibroin is soluble in acetic acid and it keeps its natural triple helix structure at 4 °C.

The blends of collagen and silk fibroin are the mixtures of two polyelectrolytes with opposite charges. Hence, electrostatic bonds are the dominating interactions in the mixtures. In the blendswithout adding the dilution solvent, the reduced viscosity is higher than silk fibroin and pure collagen which is due to the electrostatic interactions and formation of complex electrostatic networks that are coupled with conformational change of silk fibroin from silk I to silk II. These results are compatible with previous results obtained from DMA, DSC, TGA, and FTIR. Also with adding the dilution solvent the reduced viscosity ofall systemsis diminished.

Overall, the attained results conclude that viscometry is a definite method that leads to mix oppositely charged polyelectrolytes properly and uniformly and prevent any phase separation.

The XRD results show that all the mixtures have the same XRD trend as silk fibroin.The achieved results for XRD are not totally accurate since the films arevery thin and more detailed assessments need more precise analysis such as GIXRD (glancing incidence X-ray diffraction, WAXS (wide angle X-ray) and SAXS (small angle X-ray) which can specifically analyse the interactions of macromolecules in thin films.

Future collaboration with the host institute

Both institutes are eager to have collaboration work regarding more details in molecular interaction of the two biopolymers before and after dialysis, with other equipment such as WAXS, SAXS, Rheology and AFM. Different studies on the complexes formed by these mixtures before and after dialysis and the influence of different parameters such as salt, pH, and polymer concentrations

are of future interests of these groups. Additionally, we are planning to introduce new joint projects on these biomaterials with the aim of bone and skin regeneration and also drug delivery. We are just preparing a proposal for National Science Centre in Poland under HARMONIA program. HARMONIA is a funding opportunity designed for scientists wanting to carry out research in the following forms: 1) in cooperation with foreign partners, 2) within the framework of international programmes or initiatives announced under bi- or multilateral cooperation. Evaluation criteria are the following: scientific excellence and the innovative nature of the research project, the research portfolio of the Principal Investigator and his or her international partner, added value through international cooperation The deadline is 15 September 2014. Two strong research teams who have experience in collaboration under COST Action MP1301 will have great potential to apply for the next grant under the Frame Programmes of the European Commission. We will do our best to prepare proposal to EC under HORIZON2020.

Foreseen publications/articles to result from the STSM

From this collaboration, one paper on "phase behavior and miscibility studies in Collagen/Silk Fibroin macromolecular system" is under preparation for submission.

Confirmation by the host institution of the successful execution of the STSM

Dear Dr. Francis Cambier
Chair of COST Action MP1301

Ima Ghaeli spent one month in June 2014 in Faculty of Chemistry Nicolaus Copernicus University, Torun, Poland under STSM supported by COST Action MP1301. During her stay she has been working with collagen and silk fibroin materials. She prepared collagen/silk fibroin blends by mixing different ratios of both components. Miscibility of the system was studied by viscometry technique. The viscosity behaviour of aqueous mixtures formed by collagen and silk fibroin was investigated at 25 °C by Ubbelohde capillary viscometer. The intrinsic viscosity and the viscosity interaction parameter of each polymer solution as well as the mixtures were determined and served for estimation of the miscibility of different polymer mixtures by classical dilution method. From blending solutions thin films were obtained and XRD measurement has been done. The results obtained are very promising and can be published in scientific journal.

During her stay in my research group Ima Ghaeli worked very hard and during this short stay she scheduled and carried out all experiments herself. In one month she managed to work with viscometry techniques and suitable calculations. Always, in her work, Ima was consistent, dedicated, passionate, enthusiastic, cheerful, and it was a pleasure to work with her. In the lab she has been working with scientific imagination.

In my opinion Ima Ghaeli executed this STSM with success and for sure she will publish the results in near future.

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