Silver nanoparticles' incorporation in spider silk (*Paraphidippus aurantius*) for therapeutic purposes

Incorporación de nanopartículas de plata en seda de araña (*Paraphidippus aurantius*) con fines terapeúticos

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Abstract

Spiders produce a wide range of multipurpose silk fibers that are composed of Fibroin (Xiaonan *et.al*., 2016) with hydrophilic, biodegradable, biocompatible and high-strength properties. On the other hand, silver nanoparticles have remarkable properties, and their morphology can be controlled and strongly influenced by the experimental conditions, generating an effect on their antimicrobial capacity. The present work proposes a successful experimental sequence for the incorporation process of Silver Nanoparticles synthesized from Aloe Vera extract in Spider (*Paraphidippus aurantius*) silk. Once the spider web is clean and sanitized, it is submerged in a solution of Silver Nanoparticles (Solomon et al., 2007), to be subjected to sonication in order to achieve incorporation.

Silver Nanoparticles, Spider Silk, Therapeutic, Incorporation, Nanoparticles, Biocompatible

Resumen

Las arañas producen una amplia gama de fibras de seda multipropósito que están compuestas por Fibroina (Xiaonan et.al, 2016) con propiedades hidrofílicas, biodegradables, biocompatibles y con alta resistencia. Por otro lado, las nanoparticulas de plata tienen propiedades notables, y su morfología puede ser controlada y fuertemente influenciada por las condiciones experimentales generando un efecto en su capacidad antimicrobiana. El presente trabajo propone una secuencia experimental exitosa para el proceso de incorporación de Nanopartículas de Plata sintetizadas a partir de extracto de Aloe Vera en seda de Araña (*Paraphidippus aurantius*). Una vez que la tela de araña se encuentra limpia y sanitizada, es sumergida en una solución de Nanopartículas de Plata (Solomon et al., 2007), para ser sometida a sonicación con el fin de lograr la incorporación.

Nanopartículas de Plata, Seda de araña, Terapéutico, Incorporación, Nanopartículas, Biocompatibilidad

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1. Introducción

The *Paraphidippus aurantius* spider, also known as the green jumping spider, is an arachnid belonging to the family *Salticidae* of the order Araneae. This species received the name of *Salticus aurantius* and was described by Lucas (1833), the presence of this species has been identified in the southeastern United States, in Mexico, Central America and Colombia (Richman, et al., 2012). In the case of our country, it has been observed in the states of San Luis Potosí, Tamaulipas, Jalisco, Guanajuato, Colima, Michoacán, Guerrero, Tlaxcala, Puebla, Veracruz, Oaxaca and Chiapas.

The use of spider silk as an alternative for the treatment of wounds is a practice in use since ancient times, it is known that, as part of the first-aid kits for the wounded of ancient European armies, spiders were transported in small boxes to obtain silk from them even on the battlefield. The hemostatic properties of spider webs were described by Pliny the Elder about 2000 years ago (Calafat, 2010).

The silk of this spider, like that of other species, is considered a natural biopolymer, which due to its progressive and complex structure and to its physical and chemical properties is of special interest, given the fact that it is a protein formed in the glands of the spider's body, used by them for numerous purposes such as: transportation, courtship, shelter and prey capture (Kiseleva *et. al*., 2020).

This silk allows to absorb more energy before breaking than any synthetic material in common use, because these fibers have a protein structure, with hydrophilic properties, biodegradable, biocompatible with high resistance, this is owing to the fact that the silk of the spider web is covered with fungi that contain antibiotics to prevent other microorganisms from eating the fabric (Plaza, 2005). Silk threads are characterized by high tensile strength comparable to Kevlar and increased flexibility that makes spider silk unique among natural and man-made materials.

Spiders manufacture exceptionally developed silk fibers with a colossal functional capacity of the material through controlled assembly of proteins called spidroins into specific fibers of up to 7 different types (Xiaonan *et.al*., 2016).

During the processing of spider silk under changes in temperature, pressure, and pH in a narrow spinning, the spidroins organize themselves into an ordered nanocomposite fiber with a complex microstructure.

According to Vollrath (2000), the mechanical properties such as elongation, elasticity, toughness and Young's modulus reported for spider silk in fibers with moderately low diameters (100 µm) compared to silkworm threads that reach a maximum diameter of 25 micrometers. In addition to its mechanical properties, the high biocompatibility and controlled biodegradability of spider silk deserve special mention (Allmeling, *et al*., 2007).

The combination of these unique characteristics make spider silk a very attractive fiber for applications in medicine, protective materials and textiles, since it is possible to produce a stable and compact structure, resistant to degradation under human physiological pH.

These silks are hygroscopic polymers because of the presence of polar residues in the proteins that constitute them and the water acts on them as a plasticizer that makes them flexible (Plaza *et. al*., 2005).

Furthermore, as the hierarchical arrangement of spider silk fibers is possible through intra- and intermolecular protein interactions (Troncoso, 2014), it implies that these microarchitectures can be used to incorporate inorganic segments into silk filaments to deliver mineral and functional loads additional to biopolymer hybrids.

Therefore, there is currently a window of opportunity to modify silk with various inorganic components at the nanoscale, such as metal and metal oxide nanoparticles (NP´s), inorganic salts, and carbon-based nanomaterials.

Silk fibroin is a favorable biomaterial for bone tissue engineering, and silver nanoparticles (Ag NP´s) show antimicrobial activity against a large number of microorganisms, including antibiotic-resistant strains (Patil & Singh, 2019; Pedro, *et. al*., 2022). A silk fibroin scaffold with Ag NP´s and its antimicrobial properties can be considered a tissue for advanced applications without compromising its cytocompatibility and stem cell differentiation potential. (Patil & Singh, 2019).

It is well known that Ag NP´s have remarkable properties, moreover, their applications are microelectronic, chemical, mechanical in industry, pharmaceutical and biomaterial (Hagura, *et. al*., 2010). The morphology of Ag NP´s can be controlled and strongly influenced by the experimental conditions in order that the antimicrobial effect depends on the concentrations. Consequently, if the concentrations increase the area of antibacterial activity increases as well. To eliminate microorganisms in low concentrations with less toxicity, plant extracts are rich sources of secondary metabolites that easily reduce silver nitrate to silver Ag NP´s. Thus, the synthesis of NP´s by the green method in which plant extract is used as a reducing agent, it is gaining importance due to its lower toxicity and harmlessness to the environment. (Khan, et al., 2017)

2. Methodology

Collection of spider silk

Spider silk was collected within the ecological reserve owned by the Fidel Velázquez Technological University, Latitude 19° 36' 47.93" Longitude -99° 20' 21.75", located in the municipality of Nicolás Romero, in the Estado de Mexico State. Once the presence of the species *Paraphidippus aurantius* was identified, the silk was collected with the help of wooden sticks. Furthermore, in order to facilitate the collection, a process of breeding some of them inside a terrarium was established. Six samples of approximately 0.5 g in weight were collected and placed in sterile vials for further processing.

Figure 1 Spider *Paraphidippus aurantius* also known as *jumping spider*
Source: Richman (2012)

Silk cleaning

Once the silk was collected, it was placed on a surface and spread out to facilitate the removal of larger residues with the help of tweezers; using the optical microscope, the largest number of smaller residues was removed. Once the elimination of residues was completed, the silk was immersed for 15 minutes in a 5% sodium hypochlorite solution under constant agitation until completing 3 washes, changing the hypochlorite solution in each case whenever it appeared cloudy.

Sterilization of silk

After the washes, the silk was placed in a convex glass and placed for 20 minutes in infrared light to accelerate its drying. The silk was placed in a 100 ml Erlenmeyer flask and autoclaved at 15 psi and 121°C for 90 minutes to dry it again in a convex glass with infrared light.

Figure 2 *Paraphidippus aurantius* spider silk drying in infrared light

Sample storage

The silk was later stored at room temperature in a sterile glass bottle. The final weight of spider silk was on average 0.1g in each processed sample.

Synthesis of silver nanoparticles (AgNP´s)

The synthesis of silver (Ag) NP´s was carried out by green chemistry using an Aloe vera extract as a reducing agent, taking 1mM AgNO3), as the initial starting solution, to obtain yellow colored colloidal dispersions of Ag, stable and transparent. Afterwards, it was prepared a solution of 1mM AgNO3.

At the moment of adding the silver salt, continuous agitation was mandatorily observed; consequently, the formation of particle aggregates began, which was manifested through a light yellow coloration (Ahmed et al., 2016).

Incorporation of silver nanoparticles (Ag NP´s) into silk

For the incorporation of the AgNP´s to the silk, it was submerged as stretched as possible inside the beaker with the 1mM $AgNO_3$ solution. Placing the silk on the surface of the beaker, the sonication process continued for 15 minutes (Flores Garcia, 2022).

Figure 3 Spider silk cleaning and sonication process

3. Results

Once the synthesis of the silver nanoparticles was carried out as proposed by Ahmed, (2016), and before being incorporated into the spider silk, the UV-Vis characterization was carried out as a way to identify if the silver was the correct diameter.

UV-Vis characterization

Ultraviolet-Visible light absorption spectroscopy (UV-Vis) is one of the most widely used techniques for the primary characterization of synthesized NPs, in addition, it is also used to control the synthesis and stability of AgNPs. It is a fast, simple technique applicable to different types of NPs. It is based on the absorption of ultraviolet and visible radiation by the analyte that creates an activated state, which subsequently eliminates its excess energy in the form of heat. UV-Visible spectroscopy is a technique that is sensitive to the presence of Ag colloids because these NPs present an intense absorption peak due to the excitation of surface plasmons.

These free electrons originate a surface plasmon resonance (SPR), which occurs due to the collective oscillation of the electrons of the AgNPs in resonance with the light wave. When the frequency of the electromagnetic field becomes resonant with the coherent movement of electrons, strong absorption occurs (producing a color change) Cruz et al., 2012).

The position and shape of this band are influenced by different factors such as the surrounding environment, the size, shape, polydispersity of the particles, and substances adsorbed on their surfaces.

As we can see in the absorption spectrum (figure 4) around 400-450 nm, this is indicative of the presence of AgNPs, as reported by Solomon and cols. (2007), in the UV-Vis spectrum, a particle size between 10-14 nm can be attributed to the absorbance between 395-405 nm, while in the absorption peak that was obtained there is a displacement at 430 nm, therefore we obtain an average size of 35- 50nm.

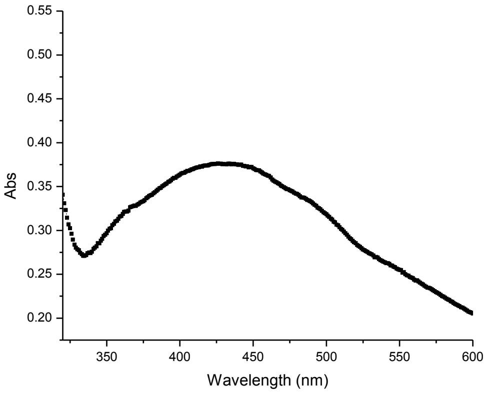

Figure 4 Uv-Visible Spectrum of Ag NPs synthesized with Aloe vera that were incorporated into spider silk

SEM characterization

The scanning electron microscope uses a low energy electron beam, this technique is used for obtaining images of the surface of the sample, providing images with great depth resolution of the focused micrograph' entire area, the following micrographs present the morphological characterization of the incorporation of AgNPs in spider silk.

Consequently. this technique allowed to analyze the surface of the samples; thus, it was possible to discern the presence of the AgNPs cluster and its influence on the spider silk.

In figure 5 the fibers of the spider silk can be seen overlapping some fibers with others, the importance about their arrangement is that it benefits and facilitates the physical incorporation of AgNPs,

In the micrograph, agglomerations of the metallic silver particles are observed on the entire surface of the spider web.

This confirms the hypothesis of our preliminary characterization of spider silk which floats in an aqueous environment, but after physical incorporation of the NPs with ultrasound it sinks and this indicates that the AgNPs were successfully incorporated, the micrograph shown in Figure 6, points out and verifies that the AgNps clusters are adhered to the spider silk fibers.

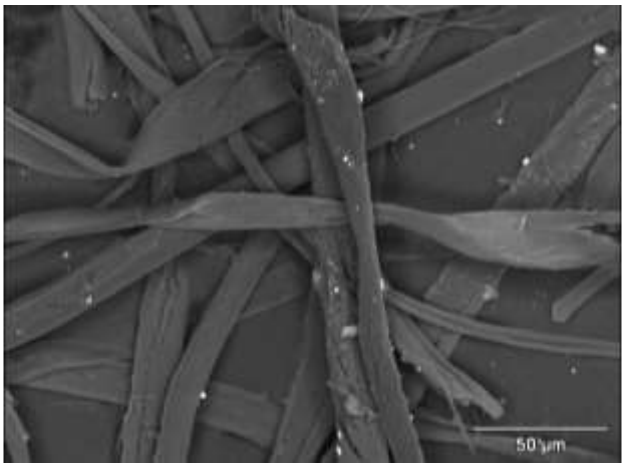

Figure 5 SEM micrograph of spider silk with the incorporation of NPsAg

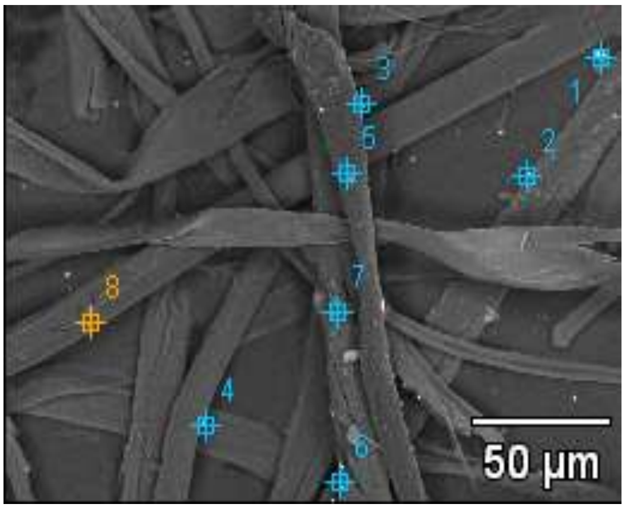

Figure 6 SEM micrograph of spider silk in the presence of NpsAg with nanoparticle dot identification

4. Conclusions

From the experimental sequence previously described, there is high feasibility for spider silk scaffolds to provide a matrix for the accommodation of silver NP´s and thus this biomaterial can be added to the list of ideal alternatives for the healing process and cicatrization for therapeutic purposes because according to studies (Patil & Singh, 2019) silk has biodegradable and non-toxic properties after implantation *in vivo* for cells to replace or repair the original tissue or organ.

This composite material (Silk-NPsAg) can be considered a promising candidate, in medicine, for tissue engineering. Moreover, considering silk fibroin as a favorable biomaterial for tissue engineering, alongside the antimicrobial activity of silver NP´s, they can be a viable alternative to combat pathogenic microorganisms, including antibiotic-resistant strains.

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