Acta Biomaterialia 45 (2016) 262–275

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Safety and tolerability of silk fibroin hydrogels implanted into the mouse brain

Laura Fernández-García [a], Núria Marí-Buyé [a,c], Juan A. Barios [a], Rodrigo Madurga [a,c], Manuel Elices [a,c], José Pérez-Rigueiro [a,c], Milagros Ramos [a,b], Gustavo V. Guinea [a,c], Daniel González-Nieto [a,b,*]

[a] Center for Biomedical Technology, Universidad Politécnica, Madrid, Spain
[b] Biomedical Research Networking Center in Bioengineering Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain
[c] Departamento de Ciencia de Materiales, ETSI Caminos, Canales y Puertos, Universidad Politécnica de Madrid, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 24 July 2016
Accepted 1 September 2016
Available online 2 September 2016

Keywords:
Silk fibroin
Hydrogels
Brain tissue
Biocompatibility
Neurophysiological evaluation

ABSTRACT

At present, effective therapies to repair the central nervous system do not exist. Biomaterials might represent a new frontier for the development of neurorestorative therapies after brain injury and degeneration. In this study, an *in situ* gelling silk fibroin hydrogel was developed via the sonication-induced gelation of regenerated silk fibroin solutions. An adequate timeframe for the integration of the biomaterial into the brain tissue was obtained by controlling the intensity and time of sonication. After the intrastriatal injection of silk fibroin the inflammation and cell death in the implantation area were transient. We did not detect considerable cognitive or sensorimotor deficits, either as examined by different behavioral tests or an electrophysiological analysis. The sleep and wakefulness states studied by chronic electroencephalogram recordings and the fitness of thalamocortical projections and the somatosensory cortex explored by evoked potentials were in the range of normality. The methodology used in this study might serve to assess the biological safety of other biomaterials implanted into the rodent brain. Our study highlights the biocompatibility of native silk with brain tissue and extends the current dogma of the innocuousness of this biomaterial for therapeutic applications, which has repercussion in regenerative neuroscience.

Statement of Significance

The increasingly use of sophisticated biomaterials to encapsulate stem cells has changed the comprehensive overview of potential strategies for repairing the nervous system. Silk fibroin (SF) meets with most of the standards of a biomaterial suitable to enhance stem cell survival and function. However, a proof-of-principle of the *in vivo* safety and tolerability of SF implanted into the brain tissue is needed. In this study we have examined the tissue bioresponse and brain function after implantation of SF hydrogels. We have demonstrated the benign coexistence of silk with the complex neuronal circuitry that governs sensorimotor coordination and mechanisms such as learning and memory. Our results have repercussion in the development of advances strategies using this biomaterial in regenerative neuroscience.

1. Introduction

In the field of regenerative engineering, the use of different biomaterials has introduced a new approach to exploit opportunities in the treatment of different human disorders in which cell death and/or degeneration constitute a common factor [1,2]. For example, the development of different biomaterials to support the function and assembly of different types of stem cells and progenitors has made substantial progress in cardiac tissue engineering [3]. When combined with cells, biomaterials should create an adequate microenvironment that can support the growth, retention and function of engrafted cells as well as the production of extracellular matrix components and other soluble factors that ensure the functional integration of the graft in the host tissue.

Brain damage by mechanical trauma or ischemic stroke and several degenerative disorders, such as Alzheimer's, Huntington's or Parkinson's disease, represent the main causes of neurological

* Corresponding author at: Experimental Neurology Laboratory, Center for Biomedical Technology, Universidad Politécnica de Madrid, Campus de Montegancedo S/N, Pozuelo de Alarcón, 28223 Madrid, Spain.
E-mail address: daniel.gonzalez@ctb.upm.es (D. González-Nieto).

http://dx.doi.org/10.1016/j.actbio.2016.09.003
1742-7061/

dysfunction in humans. Effective therapies to promote substantial recovery after brain injury are currently not available. Transcranial stimulation, optogenetics, low light laser therapy, stereotactic radiotherapy and magnetic resonance-guided focused ultrasound represent a relatively recent group of technologies for therapeutic opportunities in this field. Very recently, enhancing plasticity at the brain and spinal cord levels using antibodies against neurite-growth-inhibitory protein (Nogo-A) has been associated with improved functional recovery [4]. Another group of strategies is based in the use of stem cells encapsulated in different biomaterials, such as matrigel [5], PLGA[6], alginate [7], collagen [8] or hyaluronic acid [9], which have been demonstrated to improve functional recovery in rodents after brain injury.

The invasiveness of intra-cerebral stem cell transplantation with distinct biomaterials and their increasingly widespread use in preclinical models raises questions with respect to the use of these strategies in patients. However, this approach provides a more direct route for cell-to-cell interactions and the exact location of the implant in the brain parenchyma to support the trophic effects associated with stem cell function. Using adequate materials that can produce minor adverse effects compared with the dysfunction caused by the disease itself may compensate for the aggressive character of this procedure and justify this type of therapy, especially in patients with more severe neurological deficits.

Silk fibroin (SF) meets with most of the standards of a biomaterial suitable for the above-mentioned applications. Compared with other biomaterials, such as collagen or polylactic acid, silk induces a lower inflammatory response [10]. SF has been used in a variety of biomedical applications [2,11] and in different formats; specifically, it has been widely employed as medical sutures, for bone and cartilage tissue engineering [12,13] or recently in clinical trials for the reconstruction of the tympanic membrane and breast implants [14,15]. Although this biomaterial has never been used in preclinical models of brain repair, SF composites have been employed as anti-epileptic drug carriers [16] and in the regeneration of peripheral nerves in rats as nanoparticles or in fibrilar structures [17,18]. Due the emerging potential of SF for brain therapeutic opportunities and to the possibility for getting SF in the format of hydrogel [19], we examined the feasibility of the *in situ* gel formation induced by ultrasound sonication [20] and the short and long-term biocompatibility of this biomaterial within the mouse brain parenchyma in this study. Our results highlight the proper compatibility of this material with brain tissue. Cell death and the inflammatory responses were transient, and we did not detect sensorimotor or cognitive deficits of relevance examined by several behavioral tests and electrophysiological recordings. Our study creates a precedent for the extended use of native SF or modified forms thereof for brain transplantation in animal models as initial phases for the development of more advanced strategies in experimental brain damage and degeneration.

2. Material and methods

Other methods can be found in Supplementary material (available on the Acta Biomaterialia Web site).

2.1. Silk fibroin extraction

Silk fibroin (SF) was extracted from *Bombyx mori* cocoons, kindly provided by J. L. Cenis (IMIDA, Murcia, Spain). Cocoons were initially cut in small pieces and degummed to remove sericin in sodium carbonate solution at 0.2% (w/v) in an autoclave (121 °C, 103.4 kPa, for 30 min plus 20 min drying). After degumming, fibroin fibers were repeatedly rinsed in distilled water and allowed to dry overnight. This degumming process was shown to remove sericin from the silk fibers below values detectable by polyacrylamide gel electrophoresis [21]. Afterwards, the dry fibers were dissolved in 9.4 M lithium bromide aqueous solution for 4 h at 60 °C under continuous stirring, as previously described [22]. The final solution was dialyzed against water for 48 h using Slide-a-Lyzer cassettes (MWCO 3.5 K, Pierce). Finally, the resulting solution was centrifuged at 5000 rpm for 20 min at 4 °C to remove impurities, frozen at −80 °C and lyophilized to obtain a final SF powder.

2.2. Sonication-induced silk fibroin hydrogels

To explore the best sonication conditions to induce SF gelation, 6 ml of SF at 1 or 2% (w/v) concentration in phosphate buffer saline (PBS) without calcium and magnesium were filtered with 0.2 μm filters and introduced in centrifuge tubes. The solutions were sonicated with a Branson 450 Sonifier coupled to a 3 mm diameter Tapered Microtip. During sonication, the sample tubes were always kept in ice water. The solutions were subjected to one pulse of 30 s or two pulses of 30 s each – with 1 min pause interval between them at room temperature – at three sonication amplitudes (20%, 40% and 60%). The gelation time point was fixed at the time the liquid no longer flow when tilting the tube.

2.3. Mechanical testing

Unaxial unconfined compression tests were performed on SF hydrogels. After sonication, SF solution was poured into cylindrical molds of 10.4 mm diameter and allowed to gel at room temperature. After 24 h, gels were cut in approximately 10 mm height cylinders and placed between two parallel plates adapted to an Instron 4411 testing machine. The cylinders were centered with the loading axis of the testing machine. The force was measured with an electronic balance (Kern PLI 3500) placed under the lower plate and the compression speed was fixed at 1 mm/min. The compression tests were performed both in air (24 °C and 45% relative humidity) and with the gel sample immersed in PBS (24 °C). The cross-sectional areas were used to compute stress-strain curves from force-displacement. At least two samples for each condition were tested. Results were presented as (engineering) stress- (engineering) strain plots. (Engineering) stress, s, is defined as $s = F/A_0$, where F is the instantaneous force and A_0 the initial cross sectional area. (Engineering) strain, e, is defined as $e = \Delta H/H_0$, where ΔH is the decrease in height of the sample and H_0 is the initial height.

2.4. Monitoring the gelation process

To follow the gelation process, turbidity changes were monitored with an UV/Vis spectrophotometer (ELX808, BioTeK). Additionally, the formation of β-sheet was followed over time by attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR). For this purpose, 50 μl of solution were frozen at set time-points after sonication and subsequently lyophilized and analyzed by ATR-FTIR in a Nicolet iS5 FTIR spectrometer with an ATR module. ATR-FTIR spectra were obtained in the range of 550–4000 cm^{-1} with 64 scans per spectrum with a resolution of 4 cm^{-1}.

2.5. Animals

In vivo experiments were conducted using adult male C57BL/6 mice (20–28 g body weight; eight-ten weeks old). In a set of experiments, Alzheimer's disease transgenic mice (5XFAD) co-expressing a total of five familial Alzheimer's disease mutations, three in the human amyloid precursor protein and two in the human presenilin 1 gene were used [23]. Transgenic 5XFAD mice were obtained commercially from The Jackson Laboratory (strain