European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 139–142

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Nerve conduit scaffolds for discrete delivery of two neurotrophic factors

Stefan Catrina, Bruno Gander, Srinivas Madduri *

Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland

ARTICLE INFO

Dedicated to Hans Peter Merkle on the occasion of his 70th birthday

Keywords:
Growth factors
Controlled release
Delivery systems
Nerve conduits
Collagen
Silk fibroin
Nerve repair
Axonal regeneration

ABSTRACT

Axonal repair and regeneration remain critical due to lack of appropriate delivery systems for efficient release of neurotrophic factors (NTFs). Recently, we have demonstrated the synergistic activity of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) on axonal regeneration. Combined delivery of GDNF and NGF with individually controlled release kinetics may be crucial for exploiting their synergistic action on axonal elongation in animals. For engineering discrete NTF release kinetics, we have developed several nerve conduits (NCs) using collagen (Col) and silk fibroin (SF); the NC were made of Col or SF alone, or of Col and SF layers, or of Col/SF blends, all loaded with GDNF and NGF. All NC types provided sustained combined release of NGF and GDNF over 28 days. NC made of combinations of Col and SF showed reduced burst and more sustained dual release of GDNF and NGF. SF/Col-based NC scaffolds provide an adaptable delivery system for growth factors and hold potential for nerve regeneration and possibly for other tissue engineering applications.

1. Introduction

Peripheral nerve injuries often result in long-term motor and sensory impairment due to the problems associated with currently available treatment methods, i.e., end-to-end direct suturing, transplantation of autografts/allografts, or artificial nerve conduits (NCs) [1–5]. For improving nerve regeneration, artificial NC made various biomaterials, including collagen (Col) and silk fibroin (SF), have shown beneficial effects on peripheral nerve regeneration both in pre-clinical and clinical settings [6,7]. Nonetheless, treatment with such NC types mostly resulted in unsatisfactory functional recovery [7,8].

Strategies for improving the biological performance of NC include their enrichment with neurotrophic factors (NTFs). Yet, limited effects of NTF delivery on functional regeneration have been noted so far, which has been ascribed to aberrant peripheral target reconnections [9]. The disappointing results obtained with NTF delivery may be attributed to, e.g., suboptimal NTF concentrations, exceeding initial dose, inadequate release kinetics, inappropriate delivery site, or delivery of single rather than multiple factors.

NTFs promote neuronal survival, axonal regeneration, and Schwann cells migration [10]. NGF primarily promotes sensory neurons survival and growth [11], whereas GDNF is a highly protective factor for motor neurons [12]. Treatment with either NGF or GDNF promoted sciatic nerve regeneration in rats [13]. However, as peripheral nerves are composed of different neuronal subpopulations responsive to various NTFs [14] and because different NTFs such as NGF and GDNF afford synergistic activity [15,16], we should expect that delivery of multiple NTFs from NC is required for efficient axonal regeneration. Further, high initial NTF concentrations may cause extensive axonal branching and poor early axonal regeneration [15–17]. Therefore, we hypothesize that low initial and sustained dual release of NGF and GDNF is instrumental to avoid unwanted branching and synergizing the biological activity on axonal elongation. Development of an adaptable delivery system providing discrete release kinetics of multiple NTFs might well enhance clinical outcomes after nerve repair. For this, we have chosen SF and Col scaffolds because of their distinct slow and fast release properties, respectively [18–20]. Neither of the single material scaffolds can, however, offer discrete release kinetics for GDNF and NGF [18,19]. Therefore, engineering the delivery system by combining SF and Col scaffolds in various designs using various concentrations may provide a platform for addressing the spatio-temporal needs of GDNF and NGF for axonal regeneration.

The aim of this study was, therefore, to develop Col- and SF-based NC scaffolds that were engineered such as to achieve discrete kinetics of dual NGF and GDNF release. The NC scaffolds were built by either using Col or SF alone, or as Col-SF blends, or else by layering Col and SF; all scaffolds were loaded with both NGF and GDNF. Considering the biological benefits of low NGF and relatively high GDNF release, NGF was always premixed with SF, known for its slow release properties, and GDNF with Col, known for its faster release capacity. The formed tubes were finally coated with

* Corresponding author. Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland. Tel.: +41 (0)44 633 04 97; fax: +41 (0)44 633 13 14.
E-mail address: srinivas.madduri@pharma.ethz.ch (S. Madduri).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2013.03.030

poly(lactide-co-glycolide) (PLGA). The tubes were analyzed for porosity, swelling, thermal behavior, and NTF release kinetics.

2. Materials and methods

2.1. Materials

Bombyx mori (silkworm) cocoons were obtained from Trudel (Zurich, Switzerland) and processed in house. Insoluble bovine collagen (Microfibrillar Collagen Hemostat, Avitene) was purchased from BARD (Oberrieden, Switzerland) and poly(lactide-co-glycolide) (PLGA, Resomer RG503) from Boehringer Ingelheim (Ingelheim, Germany). Human recombinant nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) were kindly supplied by Genentech (South San Francisco, CA, USA) and Amgen (Thousand Oaks, CA, USA), respectively. ELISA reagents and antibodies were purchased from R&D Systems (Minneapolis, MN, USA). Buffer salts, solvents, and polysorbate 20 (Tween-20) were from Fluka (Buchs, Switzerland). All other reagents were purchased from Invitrogen (Buchs, Switzerland), unless stated otherwise.

2.2. Preparation of regenerated silk fibroin (SF) and collagen (Col) solutions

Cocoons from *B. mori* were boiled in 0.02 M Na_2CO_3 aqueous solution of, rinsed with ultrapurified water (UPW), and dissolved in 9 M LiBr at 55 °C to obtain a 10% (w/v) solution. The solution was dialyzed (Pierce, Rockfort, IL; MWCO 3500 Da) against UPW for 48 h. After desalination, a second dialysis against a PEG 6000 solution (200 g/1.5 l UPW) was performed to concentrate the SF solution up to 10–20%; SF concentration was determined gravimetrically [21]. Insoluble collagen (3.75%, w/w) was swollen in 0.1 M acetic acid and subsequently homogenized using high-speed homogenizer (Polytron®, Kinematica, Lucerne, Switzerland) at 12,000 rpm for 1 min.

2.3. Fabrication of nerve conduits (NCs) loaded with neurotrophic factors (NTFs)

Tubular NCs were fabricated by spinning mandrel technology. Dispersions of Col (3.7%) and SF (20%) were used individually (Col-NC or SF-NC), or deposited in layers (layered NC (L-NC)), or as blends (blended NC (B-NC)) with varying Col and SF content (Table 1). Growth factors (50 ng of GDNF and 50 ng of NGF) were dissolved in the Col or SF solution (Table 1); in case of the layered and blended NC, NGF was always dissolved in the SF solution and GDNF in the Col solution. For fabrication of layered NC, Col was used as inner layer and SF as outer layer. The mixtures were applied via a syringe onto a spinning gold-coated mandrel (diameter of 1.5 mm), installed in a sideways reciprocating apparatus, and the solvent was dried off under laminar air flow. The total amount of polymer solution applied corresponded to 25 mg/NC of dry weight. The resulting Col-NC tubes were neutralized by incubation in 0.1 M di-sodium hydrogen phosphate (pH of 7.4) for 1 h. The SF-containing tubes (SF alone, or layered with Col, or blended with Col) were subjected to water vapor treatment for inducing β-sheet formation; for vapor treatment, the tubes were placed over a saturated aqueous solution of $Na_2SO_4{\cdot}10H_2O$ (93% relative humidity) at room temperature and for 12 h [22]. The tubes were finally cut into 14 mm long specimens and spray-coated with PLGA 5% solution in chloroform.

2.4. Physical characterization of NC

NCs were characterized for porosity, swelling, and thermal properties. Porosity of the NC scaffolds was measured by gas pycnometry (Accu Pyc 1330, Micromeritics, Germany). Water uptake and swelling of NC was examined in citrate buffer (10 mM citric acid monohydrate, 150 mM NaCl, 0.05% Tween-20, pH 5.0) at 37 °C. Wall thickness and outer diameter of NC were measured before and after hydration. Water uptake of NC was measured gravimetrically over 24 h. Weight loss and glass transition temperature of NC scaffolds were analyzed by thermal gravimetry (TGA) and differential scanning calorimetry (DSC).

Table 1
Parameters involved in design and fabrication of collagen or silk fibroin nerve conduits and their layered/blended nerve conduits.

NC type	Materials		NTF loading
	Silk fibroin (%)	Collagen (%)	$GDNF_{50ng}$ + NGF_{50ng}
SF100	100	–	$SF_{GDNF+NGF}$
Col100	–	100	$Col_{GDNF+NGF}$
L-SF25Col75	25 Outer layer	75 Inner layer	SF_{NGF}/Col_{GDNF}
L-SF50Col50	50 Outer layer	50 Inner layer	SF_{NGF}/Col_{GDNF}
L-SF75Col25	75 Outer layer	25 Inner layer	SF_{NGF}/Col_{GDNF}
B-SF25Col75	25	75	SF_{NGF} + Col_{GDNF}
B-SF50Col50	50	50	SF_{NGF} + Col_{GDNF}
B-SF75Col25	75	25	SF_{NGF} + Col_{GDNF}

2.5. Measurement of NTFs in vitro release from NC

In vitro release of the NTFs was tested by incubating NC in 1 ml of citrate buffered saline (10 mM citric acid monohydrate, 150 mM NaCl, 0.05% Tween-20, pH 5.0) at 37 °C in an overhead shaker. Release medium was withdrawn at predefined time points and replaced by fresh buffer over 4 weeks, according to a previously described procedure [23]. Collected release medium was stored at −20 °C until analyzed by ELISA.

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and by Student's independent *t*-test, following Bonferroni procedure with *post hoc* multiple comparisons using SPSS (version 15.0; SPSS, Chicago, IL, USA). Values with $P < 0.05$ were considered significant.

3. Results and discussion

The slow releasing SF and faster releasing Col were combined at various concentrations and architectures for engineering dual delivery of GDNF and NGF with the discrete release kinetics.

3.1. Production of NGF- and GDNF-containing NC

The different types of NC made of Col alone (Col100) or SF alone (SF100), or of layered Col and SF (L-SF25Col75; L-SF50Col50; L-SF75Col25), or of Col/SF blends (B-SF25Col75; B-SF50Col50; B-SF75Col25) each contained 50 ng NGF and 50 ng GDNF (Table 1). The amount of loaded GDNF and NGF per mg dry weight of NC scaffold was 4 ng. NGF was always premixed with SF and GDNF with Col for controlling the respective release rates. Based on earlier observations, we expected that the different NC architectures and compositions would provide distinct and prolonged NGF and GDNF release kinetics. All NC were finally coated with PLGA to restrict outward loss of embedded NTFs.

3.2. Physical characteristics of NC

Swelling equilibrium of all NC types was achieved within approx. 2 h, and water uptake increased between 2- and 4-fold,