NAPROXEN SODIUM INCORPORATED SCAFFOLDS FOR CORNEAL TISSUE ENGINEERING

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INTRODUCTION

Corneal lesions occur as a result of corneal diseases or thermal/chemical burns and cause to severe inflammation. If the damage occured in the cornea affects large area, corneal tissue transplant is needed to regain normal function and structure of the cornea (1). Difficulties in finding the donor and the complications of transplant in procedures limit the treatment, therefore the need of corneal tissue engineering techniques revealed for treatment of corneal damages (2). In the present study, naproxen sodium (NS) loaded scaffolds were prepared using poly-lactide-co-glycolide (PLGA) and were coated with collagen or poly-l-lysine. Furthermore, in vitro characterization studies were performed on scaffolds including drug loading, drug release, surface morphology and in vitro cell culture studies.

MATERIALS AND METHODS

Materials

PLGA (50:50) (Resomer®RG 503) was purchased from Boehringer-Ingelheim (Germany). NS was a gift from Abdi İbrahim (Turkey) and all the other chemicals used were analytical grade.

Preparation of the Scaffolds

NS loaded PLGA scaffolds were prepared by emulsion freze drying method. PLGA(50:50) (10%,w/v) was dissolved in dichloromethane. Aqueous NS solution (10 %, w/v) was emulsified in PLGA solution using a homogenizer. The emulsion was poured into 5 mm diameter teflon mold, freezed at – 80 °C and freeze-dried. After 48 h, the samples were removed from the freeze dryer and placed in a vacuum oven desiccator at room temperature for 24 h. Then, the scaffolds were immersed in collagen or poly-l-lysine solutions for 30 min and vacuum dried for 24 h for coating the scaffolds.

Characterization of the Scaffolds

Surface Morphologies

The surface morphologies of the scaffolds were determined by Scanning Electron Microscopy (SEM).

Drug Loading

The scaffolds were dissolved in 5 mL dichloromethane and 10 mL simulated lacrimal fluid (SLF) were added to this solution. After 30 min ultrasonication, extraction procedure was completed and the mixture was centrifuged at 14000 rpm, for 10 min. The supernatant was separated and injected into the HPLC column to assay NS.

In Vitro Release Studies

Scaffold formulations were placed in 2 mL tubes and 1 mL of SLF (pH 7.4) was added as release medium. The tubes were immersed in a water bath (37±0.5 ^{o}C) with a horizontal shaker at 20 rpm. At various time intervals, samples of 1 mL were withdrawn and replaced with the equal volume of fresh medium. The samples were injected into the HPLC column to determine NS.

Cell Culture Studies

In vitro cytotoxic effects of the scaffolds were investigated with MTT assay. The sterilized scaffolds (0,2g/ml) were incubated at 37°C for 1, 5 and 10 days in cell culture medium (DMEM F-12 supplemented with 20% (v/v) fetal bovine serum and 1% (v/v) penicilin-streptomicin). The cells ($15x10^3$ cell/well) were seeded into 96 well plate, incubated overnight in humidified 5% CO_2 at 37°C. After removing the medium, 200 µL scaffold extract was added to each well and incubated for another 24h. Then, medium was removed and 100µl fresh medium and MTT solution (in DMEM F-12) were added to each well. Incubation was allowed for 4 h and 100 µl isopropanol-HCl (absolute ispropanol containing 0.04 M HCl) solution was added to the wells. The optic density (OD) values were obtain at 570 nm using ELISA plate reader and percentage of viability was calculated. For each MTT assay, the control cell viability was defined as 100 %. The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability (\%)} = (OD_{570}(\text{sample}) / OD_{570}(\text{control})) \times 100$$

(Equation 1)

RESULTS AND DISCUSSION

Surface Morphologies

The surface morphologies of the scaffold formulations were shown in Figure 1.

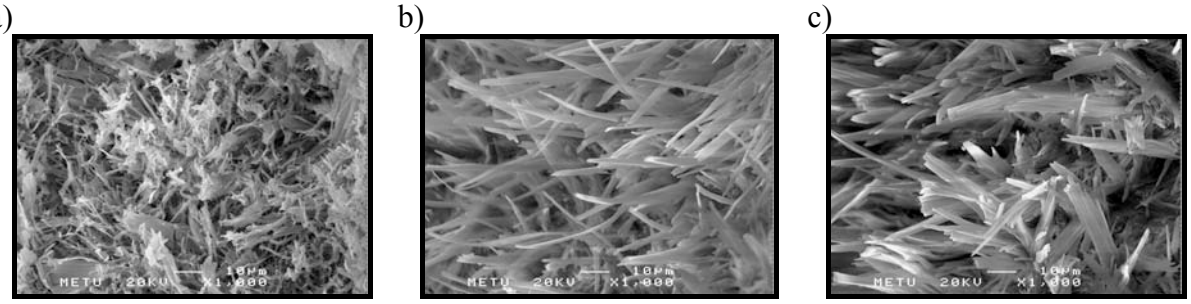

Figure 1. The surface morphogies of the PLGA scaffolds: a)uncoated, b) collagen coated, c) poly-l-lysine coated formulations.

Drug Loading
The drug loading efficiencies of the scaffold formulations were shown in Table1.

Table 1. Drug loading efficiencies of the scaffold formulations.

Formulation	Drug Loading Efficiency (%)
Uncoated	92.28 ± 2.84
Collagen coated	84.62 ± 1.78
Poly-l-lysine coated	85.82 ± 2.74

In Vitro Release Studies
The release profiles of the scaffold formulations were presented in Figure 2.

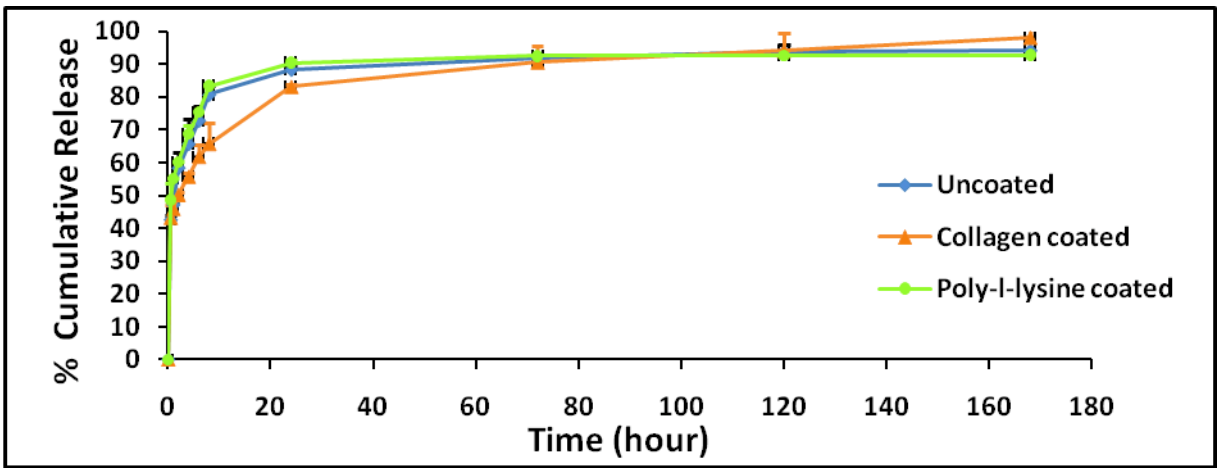

Figure 2. NS release profiles from PLGA scaffolds.

Cell Culture Studies
In vitro cytotoxicity results of the scaffold formulations on corneal epithelial cells was shown in Table 2.

Table 2. In vitro cytotoxicity of PLGA scaffolds.

Formulation	Cell Viability (%)		
	Day 1	Day 5	Day 10
Uncoated	84.5981	83.8673	84.7667
Collagen coated	88.7015	87.6897	79.7639
Poly-l-lysine coated	87.9146	87.8583	80.8881

CONCLUSION

Results indicated that PLGA scaffolds containing NS were well suited for the sustained release of NS and are promising as the carrier for drug delivery in corneal tissue engineering due to their compliance with corneal epithelial cells.

References

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