Depot Forming Dissolving Microneedles for Intrascleral Protein Delivery

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Introduction

Age-related macular degeneration (AMD) is a chronic progressing degeneration of the central retina and the leading cause of visual impairment among adults over age 65. Currently, the intravitreal injection of anti-vascular endothelial growth factor (Anti-VEGF) therapeutics is a standard approach for AMD treatment. However, owing to the chronic nature of AMD, patients require frequent injections, performed by highly invasive hypodermal needles. Therefore, the intravitreal route is always associated with severe complications, such as retinal detachment, endophthalmitis, and cataract development [1,2].

Dissolving microneedles (MNs) have been proposed as an alternative to the hypodermic needle, as a minimally invasive device to enhance patient compliance and provide localized delivery. Therefore, in this study, ovalbumin (OVA)-loaded dissolving MNs have been fabricated to transport protein to the back of the eye in a minimally-invasive manner. Several polymers have been selected and assessed for the fabrication of dissolving MNs and assessed for its characteristics to develop optimised delivery system.

Experimental Methods

Fabrication of OVA-loaded dissolving MN arrays

Dissolving MN arrays were prepared from aqueous blends of various polymers as shown in Table 1. Positive pressure was applied to force the mixture of polymers and OVA to fill the cavity of the moulds. MN mould used in this study contained 9 (3*3) conical-shaped needles, approximately 700 µm in height, with a base width of 300 µm and 50 µm interspacing. The detailed manufacturing process of the OVA-loaded dissolving MNs is presented in Figure 1.

Table 1: Different formulations of MNs

Formulation code	Polymer type and concentration (%, w/w)	OVA concentration (%, w/w)
F1	PVA 31-50 KDa/50	50
F2	PVP 58 KDa/50	50
F3	PVA 31-50 KDa/22 PVP 58 KDa/28	50
F4	HA 5 KDa/50	50

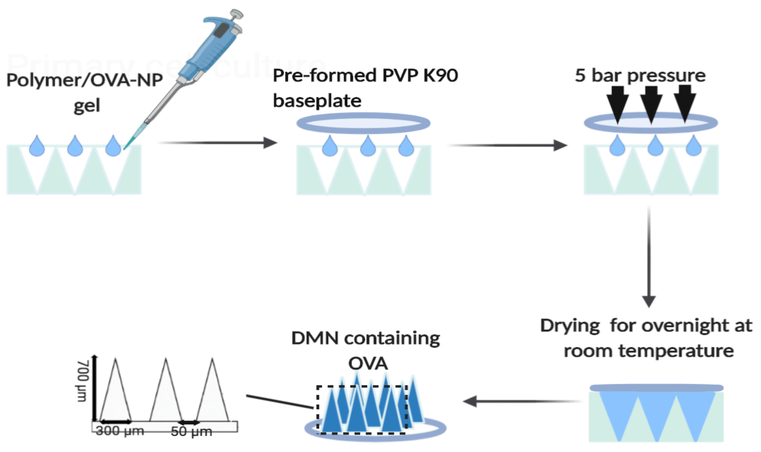


Figure 1: Schematics of the fabrication process of OVA loaded dissolving MN arrays.

Microscopy observation

The structure and morphology of various MNs were detected by Leica EZ4D digital light microscope (Leica Microsystems, Milton Keynes, UK) and scanning electron microscope (SEM) TM3030 (Hitachi, Krefeld, Germany).

Mechanical characterisation

MNs were subjected to mechanical testing using a TA-TX2 Texture Analyzer (Stable Microsystem, Haslmere, UK) in compression mode. MNs were attached to a cylindrical probe and a force of 3N was applied for 30 sec. Both MNs without drug loading and OVA-loaded MNs were visually examined pre- and post-application of the compression load using a light microscope and the heights of individual needles were measured. The percentage change in needle height was subsequently calculated.

Insertion studies

In order to evaluate the insertion ability of the MNs, the same setup was carried out and MNs were inserted into the porcine scleral tissue. The insertion depth was detected using an optical coherence tomography (OCT) (Michelson Diagnostics Ltd., Kent, UK).

Dissolution kinetics of dissolving MN arrays

MNs were inserted into porcine scleral tissue by application of thumb pressure for different time intervals (0, 30, 60, 120, 150 sec). The remaining height and percentage of OVA-loaded MN were calculated and recorded as MN height remaining vs. time.

In vitro permeation studies

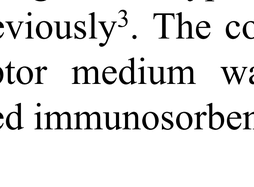
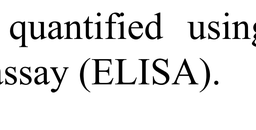
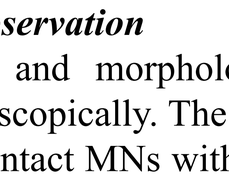
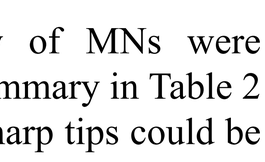
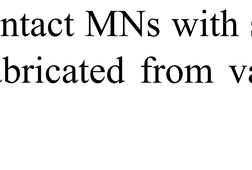
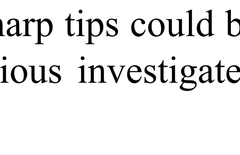
The permeation studies of different MNs were evaluated using Franz-type diffusion cells, as described previously[3]. The concentration of OVA in the receptor medium was quantified using enzyme-linked immunosorbent assay (ELISA).

Result and Discussion

Microscopy observation

The structure and morphology of MNs were detected microscopically. The summary in Table 2 indicated that intact MNs with sharp tips could be successfully fabricated from various investigated polymers.

Table 2: Structure and morphology of MN arrays fabricated from various formulations

Formulation composition	MN morphology	
50% w/w OVA 50% w/w PVA 31-50 KDa		
50% w/w OVA 50% w/w PVP 58 KDa		
50% w/w OVA 22% w/w PVA 31-50 KDa 28% w/w PVP 58 KDa		
50% w/w OVA 50% w/w HA 5 KDa		

Mechanical Characterisation

As shown in Figure 2, after compression, none of the MN tested fractured, expect HA dissolving MNs, which had the highest reduction in height (> 30%). Besides, for other groups (F1, F2, F3) after drug loading, the MN became more brittle with higher reduction after compression, while for OVA-loaded HA MNs the reduction was reduced.

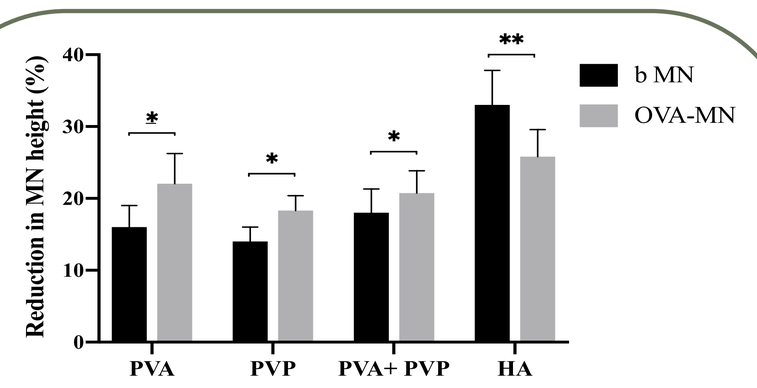


Figure 2: Comparison of percentage height reduction of non-OVA loaded MNs (b MN) and OVA-loaded MN (OVA-MN) prepared from different formulations after compression (mean ± SD, n=3).

Insertion and dissolution studies

The results of the insertion test detected by OCT were consistent with the robustness studies. Except for the HA DMNs with only 60 % depth of insertion, the MNs fabricated from all other formulations successfully penetrated porcine scleral tissue with higher insertion depth (>75%), as shown in Figure 3A and B. Besides, dissolution profiles of various types of MNs are illustrated in Figure 3C, turns out that all types of MNs could be completely dissolved in porcine scleral tissue within three minutes.

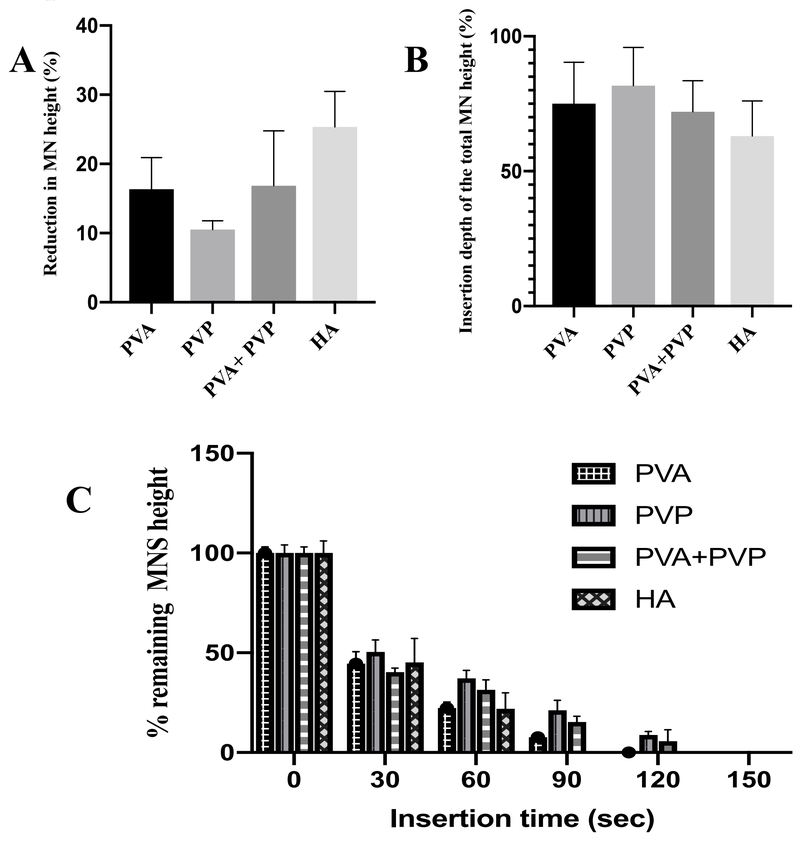


Figure 3: (A) The reduction in OVA-MN height after 3N insertion; (B) The average insertion depth of OVA-MNs; (C) Percentage of remaining MN height vs. dissolution time of OVA-loaded MNs (mean ± SD, n=3).

Ex-vivo permeation profile

Figure 4 indicates that among the investigated polymers, dissolving MNs fabricated from PVP, PVA, and PVA+PVP released approximately 50 µg of OVA in the receptor chamber, which was more than twice that of HA MNs.

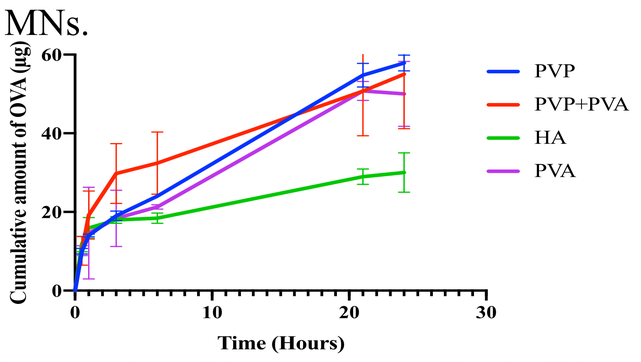


Figure 4: The comparison of the permeation profiles for OVA-loaded MNs fabricated from different polymers across porcine scleral tissue (600-700 µm) (mean ± SD, n=3).

Conclusion

This study optimised polymers to produce rapidly dissolving MN applied for posterior segment protein delivery via the intrascleral route. Except for the MN composed of HA, MNs fabricated from other polymers were sharp and robust enough to puncture the scleral tissue with limited reduction of height. *In vitro* studies indicated that MN made of all selected polymers could rapidly dissolve within the tissue after insertion and showed an increased degree of permeation of model protein, which demonstrates that dissolving MN is capable of bypassing ocular barriers and delivering high molecular weight proteins in close proximity to the target tissue (choroid/retina).

Reference

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