Structural optimization of rapidly separating microneedles for efficient drug delivery

Dan Dan Zhu, Bo Zhi Chen, Meng Chan He, Xin Dong Guo*
Beijing Laboratory of Biomedical Materials, College of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing, 100029, PR China

A R T I C L E   I N F O
Article history:
Received 26 November 2016
Received in revised form 4 February 2017
Accepted 28 February 2017
Available online xxx

Keywords:
Microneedles
Efficiency
Optimized structure
Painless
Drug delivery

A B S T R A C T
Rapidly separating microneedles (RSMNs) arose as an improvement of traditional MNs for the special separable structures. The aim of this study was to investigate various structural parameters contributed to the delivery performance of RSMNs. The experimental results indicated that the optimized RSMNs with 500 μm-long solid PLA MNs, 250 μm-long overlap and 500 μm-long dissolving MNs delivered over 95% of drugs within 30 s. In the in vivo diabetic mouse treatment, insulin loaded optimized RSMNs reached approximately the same therapeutic effect on lowering the glucose in blood as injection, significantly better than traditional MNs.

© 2017 Published by Elsevier B.V. on behalf of The Korean Society of Industrial and Engineering Chemistry.

Introduction

Compared with traditional drug delivery routes, microneedles have attracted considerable attentions for their advantages [1–6]. Micro-dimensional needle arrays enable minimally invasive delivery of diverse biomolecules including high molecular weight biologics [7–11]. These breakthroughs overcome the limitations of hypodermic needles for the potential risk of infection and plaster patches for the inability of bio-macromolecular drugs to cross skin barriers [12–14]. While dissolving microneedles meet the need of less-invasive, safe and convenient transdermal patches [15], some undesirable respects also raise increasing concerns. Due to the variation of skin elasticity, dissolving microneedles usually fail to be completely inserted into skin which leads to the inaccuracy of drug dosing and delivery efficiency [7,16–18]. Time needed for the dissolution of biologics and polymer gels (several minutes, even hours) also add inconvenience to users [19,20]. To address these issues, innovative structures of dissolving microneedles have been proposed recently [18,21,22]. By mounting water-soluble micro-needles onto metal bases, separable arrowhead microneedles are given the capability to deliver drugs into skin in seconds [23]. However, while the quick separation and high drug delivery efficiency are favorable, the left-behind metal bases after insertion still induce non-biodegradable wastes [24–27].

To develop the good functions and give up the shortcomings of separable arrowhead microneedles, we have designed rapidly separating microneedles (RSMNs) [28]. Rapidly separating microneedles are of good biocompatibility and biodegradability by selecting Polylactic acid (PLA) (3051D) instead of metal materials to fabricate supporting structures. Previous research have identified that these rapidly separating microneedles can achieve approximately 90% of drug delivery efficiency into tissues within 30 s. However, detailed studies about the structure parameters of RSMNs accounted for the drug loading and delivery efficiency are still indispensable for the following reasons: (a) There is plenty of scope to further increase the delivery performance of RSMNs by adjusting the sizes of components, such as the length of the solid PLA MNs associated with the insertion depth of RSMNs. (b) There is an increasing concern on the amount of drugs loaded in the MNs to meet high dosage [6]. Lengthening the upper dissolving MNs will contribute to the drug loading of RSMNs, (c) previous studies have reported that the degree of pain and bleeding caused by the MNs increased with the length of the MNs. The maximum allowable length of MNs were generally 1 mm [29]. Thus experiments on the optimized structure of RSMNs need to be conducted under the limits of overall length. When changing the structural parameters of RSMNs, the full length of the MNs still need to be in the shortest possible length for patient compliance (less pain without bleeding and infection) [30].

In this work, we concentrated on diverse structural parameters contributed to the insertion and separation capabilities of RSMNs. As shown in Fig. 1, RSMNs with various length of solid PLA MNs, dissolving MNs and the overlaps between dissolving MNs and solid.
PLA MNs would be fabricated to investigate the influence on the insertion depth, dissolution and separation process, and drug loadings of RSMNs, respectively. The characteristics, mechanical properties, insertion capabilities, drug loading and delivery efficiency of these RSMNs were tested to identify their properties. By evaluating the entire performance experiments, the optimum structure of RSMNs were finally determined. This is of high importance to maximize the drug loading and delivery efficiency of RSMNs for accurate drug dosing and utilized medical resources.

**Materials and methods**

**Materials**

Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) was used to fabricate the MN molds. Biodegradable polyactic acid (PLA) (3051D) was used to fabricate the solid MNs. Sulforhodamine B (Molecular Probes, Eugene, OR) was chosen as the model drug for testing experiments. PVA (polyvinyl alcohol, 75% hydrolyzed, MW2000 Da, ACROS Organics, Geel, Belgium) and sucrose (Sigma-Aldrich, St Louis, MO) were prepared to make the polymer gels for dissolving MNs. Porcine cadaver skin (Pel-Freez, Rogers, AR) was used as the skin model for penetration experiments. Streptozotocin (STZ, AbMole Bioscience) was prepared for creating streptozotocin-induced type-1 diabetic mice models. Insulin was obtained from Sigma-Aldrich Corporation (USA) to fabricate insulin-loaded RSMNs.

**Fabrication of RSMNs**

Rapidly separating MNs were made up of the drug loaded dissolving MNs and the solid PLA MNs (Fig. 2). The solid PLA MNs were firstly fabricated by casting molten PLA particles onto the 5 × 5 PDMS mold under vacuum at −85 kPa for 2 h at 200 °C. After cooled to room temperature, the PLA MNs would be peeled off from the mold. The upper drug loaded dissolving MNs were also prepared via micromolding technologies [31]. In brief, 1 mg/ml Sulforhodamine B solutions were placed onto the 5 × 5 PDMS mold under vacuum at −90 kPa for 10 min (Fig. 2a) and then, removed by pipetting and saved for further use. After 30 min drying in desicicator (Fig. 2b), the 48% (w/w) PVA/sucrose gels were cast onto the mold for 1 h (Fig. 2c). By cleaning the residual gels on the surface, various length of pasters were attached to the mold to keep the overlaps between the dissolving MNs and the PLA MNs. Then, the PLA MNs were aligned manually toward the dissolving MNs mold under a stereomicroscope (SZX7, Olympus, Japan) (Fig. 2d). After the alignment, the whole system was freeze-dried in a freeze dryer (VFD-1000, Boyikang, Beijing). And once formed, the dried quickly separating MNs were gently demoulded (Fig. 2e) and stored under vacuum for further experiments.

**Fig. 2.** Diagram of fabrication process of RSMNs (a) casting drugs onto the PDMS mold for 10 min (b) remove extra drugs on the mold surface and keep the system drying for 30 min (c) filling polymer gels in the cavities for 1 h and then removed extra gels (d) embedding solid PLA MNs onto the mold cavities (e) demoulded after the whole system freeze-dried for 24 h.
Mechanical performance test

To identify the structural stability, RSMNs were dried under vacuum for at least 24 h and then measured by a displacement-force test machine (Mark-10, Force Gauge Model, USA). Briefly, one RSMNs patch was placed on the rigid stainless steel station and pressed by a moving sensor axially at a speed of 0.1 mm/s. The force required to move the mount as a function of MN displacement was recorded.

In vitro skin penetration experiment

To testify the insertion capability of RSMNs with different structures, Sulforhodamine B loaded RSMNs were inserted into porcine cadaver skin for 30 s using a home-made applicator. Before insertion, all the RSMNs had been dried in a vacuum oven (DZF-6050, Luxi, China) at room temperature for 12 h. And the pig cadaver skin was hair-free by a razor. To facilitate the dissolution and separation of the dissolving MN from the PLA MNs, manual vibration was applied to the MN patches against the skin. After insertion, MN patches were gently peeled off and the insertion sites on the skin were exposed under a stereomicroscope (SZX7, Olympus, Japan). Moreover, to visually analyze the insertion depth of RSMNs, the pig cadaver skin was cut from the middle of the MN holes to obtain the cross-section. Front and side views of the pig cadaver skin at both bright field and fluorescence were imaged by a digital camera (Olympus DP71).

Drug loading and delivery efficiency

To determine the loading and residual amounts of RSMNs with a variety of structures, Sulforhodamine B loaded RSMN patches before and after insertion were respectively dissolved in a specific amount of DI water and then tested using a fluorescence microplate reader (Fluoroskan Ascent 374, Thermo Scientific). All the images of RSMNs before and after insertion were also taken by a stereomicroscope (Olympus SZX7).

Administration of insulin-loaded RSMNs in diabetic mice

Once the optimum structure of RSMNs were determined, insulin-loaded RSMNs were fabricated and applied to diabetic mice to verify whether the drug loading and delivery efficiency met the expectation. Female Balb/c mice, 25.5 ± 0.5 g, were fasted for 6 h and then injected 0.5 ml STZ-SCB solution (STZ powder dissolved in sodium citrate buffer, 10 mg/ml) to develop diabetes. The blood glucose levels of the treated mice were measured by tail vein laceration via blood glucose test strips (Onetouch, Shanghai). After the blood glucose (BG) level reached 300–550 mg/dl (generally within 48 h), insulin-loaded RSMNs were applied to the mice skin (n = 6, hair removed) for 30 s. Thereafter, the BG levels of diabetic mice were collected every 30 min in the next 6 h.

To persuasively illustrate the improvement of delivery efficacy of RSMNs, traditional dissolving MNs of same insulin dose were also inserted to diabetic mice (n = 6) for 30 s as the contrast experiment. The hypoglycemic effects of both RSMNs and traditional dissolving MNs were taken hypodermic injection of insulin as reference. The BG levels of mice treated with blank MNs and PBS were also collected as control. All the animal studies were carried out in accordance with the guidelines for animal experimentation, the Ethics Committee of BUCT.

Statistical analysis

All analyzes were conducted with a sample size of n ≥ 6. Data were analyzed using Excels and Origin85. All values were expressed as their mean ± SE. Statistical differences were assumed to be reproducible when p < 0.05.

Results and discussion

Rapidly separating microneedles with various length of solid PLA microneedles

The skin’s elasticity caused difficulties for MNs to be fully inserted into skin, which further led to undesirable insertions and uncontrollable drug dosages. To address these issues, solid PLA MNs of RSMNs were designed to overcome skin deformation as a mechanical spacer. Various length of solid PLA MNs generally affected the insertion depth of RSMNs into tissues, which determined the fraction of dissolving MN residues outside the skin. Apparently, the longer the solid PLA MNs were, the easier for RSMNs to achieve complete insertion. But the limitation of the length for MNs to avoid needle-induced pain and bleeding was also significant for patients’ compliance. So, to discuss the reasonable length of solid PLA MNs and examine the influences on the insertion depth of RSMNs, three groups of RSMNs with 400 μm, 500 μm and 600 μm PLA MNs were fabricated respectively. To control variables, the length of dissolving MNs was set to 500 μm and the overlap of RSMNs with various length of PLA MNs were set to 250 μm. Fig. 3A1–A3 showed the typical photos of the Sulforhodamine B loaded RSMNs with various length of PLA MNs. The amount of drugs loaded in RSMNs with various length of PLA MNs was approximately 280 ng/patch. It is obvious that the height of upper drug loaded dissolving MNs above the MN bases were increased with the incremental length of PLA MNs.

Fig. 3. Images of RSMNs with (A1) 400 μm, (A2) 500 μm and (A3) 600 μm-long solid PLA MNs before (A1, A2, A3) and after insertion (B1, B2, B3). Side views of pig cadaver to observe the insertion depth of RSMNs with (C1) 400 μm, (C2) 500 μm and (C3) 600 μm-long solid PLA MNs. (D) The percentage of drugs delivered into the skin by RSMNs with various length of overlaps.

To identify the insertion depth of RSMNs with various length of PLA MNs, RSMNs fabricated through the above method were applied to hair cleaned pig cadaver skin for 30 s. After insertion, the MN patches were then removed and observed under a stereomicroscope. As shown in Fig. 3B1, there were quite a few drug residues remained on the 400 μm-long solid PLA MNs. As for RSMNs with 500 μm and 600 μm-long PLA MNs, less drugs were left on the patches (Fig. 3B2 and B3). To further visualize the depth of drug loaded dissolving MNs embedded in the tissues, the insertion sites of pig cadaver skin were sliced from the middle of the microholes. As shown in Fig. 3C1–C3, the insertion depth of RSMNs with 400, 500 and 600 μm-long solid PLA MNs was approximately 420, 530 and 630 μm, respectively. These experimental phenomena proved that the length of the solid PLA MNs directly influence the insertion depth of the upper dissolving MNs.

Due to the skin elastic deformation, RSMNs with shorter length of PLA MNs (400 μm, Fig. 3C1) failed to completely embed the drug loaded dissolving MNs into the tissues and led to the incomplete dissolution. For the two other RSMNs with 500 μm and 600 μm-long PLA MNs, the length of PLA MNs exposed outside the dissolving MNs were approximately 250 μm and 350 μm respectively. During the insertion, the exposed 250 μm and 350 μm-long PLA MNs successfully overcome the skin dimpling. Thus these results qualitatively indicated that the length of the exposed part of PLA MNs were supposed to be no shorter than 250 μm to fill up the depression of skin, which contributed to the complete insertion of the upper dissolving MNs.

To statistically demonstrate the amount of drug delivered by RSMNs with various length of PLA MNs, the removed MN patches after the insertion were collected and several dissolves in a specific amount of DI water, then quantified using a fluorescence microplate reader. And the calculated drug delivery efficiency of RSMNs with various length of PLA MNs was shown in Fig. 3D. For the RSMNs with 400 μm-long PLA MNs, drugs remained on the patches disappointingly accounted for approximately twenty percent of drug loadings. As for the RSMNs with 500 and 600 μm-long PLA MNs, both drug delivery efficiencies successfully reached up to more than 95%. These results were consistent with the preceding qualitative conclusions, that RSMNs with 500 and 600 μm-long PLA MNs were more suitable for drug delivery when compared with RSMNs with 400 μm-long PLA MNs. Since the drug delivery efficiencies of RSMNs with 500 and 600 μm-long PLA MNs were slightly different from each other and to keep the full length of RSMNs as short as possible (for better patient compliance, as mentioned in the introduction), the length of solid PLA MNs was ultimately set to 500 μm for forthcoming experiments and practical applications.

Rapidly separating microneedles with various overlaps between dissolving MNs and solid PLA MNs

Traditional MNs generally took minutes even hours to fully dissolve into the skin, which added inconvenience for users to receive treatments. Also it was difficult to precisely control the drug dosages through uncertain duration of MNs’ application, which might further cause unpredictable consequences. To shorten the time needed for MN administration and deliver the most of loaded drugs, the mosaic structure of RSMNs was designed to transform the MN administration process to the dissolution and separation of dissolving MNs from solid MNs and greatly decreased the time needed for MNs applications. In principle, the shorter the length of the overlap was, the less the time spent on the dissolution and separation. Nonetheless, if too short of the overlap was set, the RSMNs would be brittle and prone to breaking due to the bad structural connection between dissolving MNs and solid PLA MNs. Thus, to determine the proper length of the overlaps, RSMNs with various overlaps were fabricated to evaluate their structure stability and insertion capability. The length of dissolving MNs and solid PLA MNs were set to 500 μm as the control variables. To ensure the various length of overlap, the thickness of plasters was set to 150, 250 and 350 μm (i.e. the corresponding overlaps were 350, 250 and 150 μm respectively). Fig. 4A1–A3 showed the RSMNs with different overlaps. The amount of drugs loaded in RSMNs with various length of the overlaps was approximately 280 ng/patch.

To testify the influence of various overlaps on the structural stability and insertion capability of RSMNs, the mechanical failure test was firstly conducted. As shown in Fig. 4B, the curves of RSMNs with 350 and 250 μm-long overlaps were similar. They both

Fig. 4. Images of RSMNs with (A1) 350 μm, (A2) 250 μm and (A3) 150 μm-long overlap before (A1, A2 and A3) and after insertion (C1, C2, C3). Front views of the pig cadaver to observe the insertion of RSMNs with (D1) 350 μm, (D2) 250 μm and (D3) 150 μm-long overlap. (B) Mechanical performance of RSMNs with various length of overlaps. (E) The delivery efficiency of RSMNs with various length of overlaps.

showed a slow initial increase and then grew rapidly without reaching a distinct transition point. Previous studies confirmed that the insertion force needed for one MN was less than 0.1 N, which indicated that RSMNs with 350 and 250 μm-long overlaps were theoretically capable of penetrating the skin without being broken. As for RSMNs with 150 μm-long overlap, the curve suddenly dropped at the displacement of 0.2 mm as the result of the MN breakage, indicating the undesirable mechanical strength caused by the too-short length of overlap.

Skin insertion experiment was further launched to verify the mechanical and drug delivery performance of RSMNs with various overlaps. As shown in Fig. 4D1 and D2, RSMNs with 250 and 350 μm-long overlaps achieved complete insertion into the pig cadaver. The 5 × 5 micro-holes on the skin surface were clearly visible. And fewer drug residues were left on the PLA MNs (Fig. 4C1 and C2). However, for RSMNs with 150 μm-long overlap, there were quite a few broken needles remained on the patches after insertion (Fig. 4C3). Only part of drugs loaded in the upper dissolving MNs were delivered into the tissues (Fig. 4D3). To statistically analyze the drug delivery efficiency, the RSMNs after insertion were dissolved in a specific amount of DI water to determine the fluorescent intensity. Fig. 4E intuitively illustrated the drug delivery efficiency of RSMNs with various overlaps. Within 30 s, RSMNs with 250 μm and 350 μm-long overlap delivered approximately 95% and 87% drugs into the skin respectively. The delivery efficiency of RSMNs with various overlaps was decreased with the increasing length of the overlap, which could be attributed to the longer time needed for dissolving MNs to dissolve and separate from the solid MNs. But for RSMNs with 150 μm-long overlap, the delivery efficiency plunged to 27%.

These results were consistent with the inference that various length of overlaps between the dissolving MNs and the solid PLA MNs affected the drug delivery condition of RSMNs. Shorter length of overlaps contributed to easier dissolution and separation process. But too-short connections between dissolving MNs and solid PLA MNs led to the imperfect mechanical strength of RSMNs, which resulted in unsatisfactory drug delivery performance. Thus to obtain better insertion capability and deliver maximum drugs in limited time, the optimal overlap length of RSMNs were approximately set to 250 μm.

### Rapidly separating microneedles with various length of dissolving MNs

The amount of drug loaded in MNs were generally restricted by the drug formulations, the shape and the length of the MNs. Normally, by increasing the length of the dissolving MNs, the drug loadings could be easily controlled to meet higher drug dosages. However, previous studies suggested that the length of the MNs needed to be less than 1 mm to avoid significant pain and bleeding. But it’s vital to note that the changing length of the needle might impact on the insertion ability and drug delivery performance of MNs. Thus to assess the drug loading capacity and delivery efficiency of the RSMNs with various length of dissolving MNs, RSMNs with 400, 500 and 600 μm-long dissolving MNs were made. The length of the solid PLA MNs was set to 500 μm and the overlap was set to 250 μm.

By separately fabricating the solid PLA MNs, the dissolving MNs and then assembled them, RSMNs with various length of dissolving MNs were demoulded after drying (shown in Fig. 5A1–A3). The insertion of RSMNs with various length of dissolving MNs were investigated in pig skin after the drying process. As shown in Fig. 5C1–C3, the insertion sites were evaluated under microscope. These successful insertions proved that the length of dissolving MNs were not critical for the mechanical strength of RSMNs. RSMNs with 400, 500 and 600 μm-long dissolving MNs were all capable of delivering drugs into the tissues. And after insertion, the removed MN patches were gathered up to observe the residues. In Fig. 5B1–B3, few drugs remained on the used patches of RSMNs with 400, 500 and 600 μm-long dissolving MNs. To better demonstrate the drug loading capacity and drug delivery efficiency of RSMNs with various length of dissolving MNs, the MN patches after insertion were dissolved to determine the fluorescent intensity. The statistical data in Fig. 5D showed that RSMNs with 400, 500 and 600 μm-long dissolving MNs all scored well on performance of drug delivery, with delivery efficiencies surpassing 90%. By adjusting the length of the dissolving MNs, the amount of loaded drugs could be accurately controlled. When the length of dissolving MNs was set to 400, 500 and 600 μm, the amount of drug loaded in one RSMN was approximately 8.62, 11.27 and 14.02 μg (shown in Fig. 5D), respectively. These experimental results indicated that the upper dissolving MNs rarely affected the insertion ability and drug delivery performance of RSMNs. The tips of the upper dissolving MNs corners kept sharp when the length increased. Thus, the length of the upper dissolving MNs depended mainly upon the demand of drug dosages. For RSMNs, based on the aforementioned studies, the length of the dissolving MNs needed to be less than 750 μm when the length of the solid PLA MNs and the overlaps were set to 500 and 250 μm.

### In vivo study of rapidly separating microneedles in diabetic mice

Based on the above study, to maximize the improvement of the drug delivery efficiency of RSMNs and simultaneously keep the
total height of RSMNs as short as possible, the rational length of the solid MNs and the overlaps were ultimately selected as 500 and 250 μm, respectively. As regards the upper dissolving MNs, the length contributed to the amount of drugs loaded in the RSMNs and had little effect on the delivery efficiency. In a follow-up in vivo experiment to treat diabetic mice and in consideration of drug formulations, 500 μm-long dissolving MNs were chosen to fabricate the insulin-loaded RSMNs (i.e. the total height of RSMN is 750 μm).

To practically test the drug delivery performance of the optimized structure of RSMNs, the optimized RSMNs (Fig. 6A) were loaded with 0.1 U insulin to treat diabetic mice. Female Balb/c mice were divided into 3 groups (6 in each) and treated with the optimized RSMNs, traditional MNs and injection at the insulin dose of 0.1 U. For comparison, another 2 groups of mice were treated with blank MNs and injection of PBS solution. After the insulin treatment, the BG level of mice was monitored and recorded every 30 min during the next 6 h. As shown in Fig. 6B, all the BG level curves shared a general character of decreasing firstly and then increasing. For the injection group, the blood glucose level declined immediately and reached the minimum at 1.5 h, which was approximately 20% of the initial. For the other 2 groups receiving microneedle patches, the blood glucose responses slightly lagged behind and the BG level decreased slowly in the initial 30 min, which could be due to the time needed for the dissolution and release of insulin loaded in MNs. Then, the curves of mice treated with the optimized RSMNs and the traditional MNs both reached minimum around 2 h after the administration of MN patches. The drop of the BG level of the optimized MNs group, approximately 82% of the initial, was similar to the injection group and much larger in magnitude compared with the traditional MNs group, which was only 64% of the initial. These results suggested that the optimized RSMNs successfully delivered almost all loaded insulin within 30 s, which could be attributed to the improved microstructure. But for traditional MNs, due to the skin deformation, the needles failed to be fully inserted into skin which led to the insufficient drug dosage. In consequence, the optimization of the RSMNs’ structure maximize the drug delivery efficiency of RSMNs, which effectively reduce the time needed for MN administration and contributed to the accurate drug dosing.

Conclusion

As described elsewhere, traditional MNs generally take many minutes or even hours to slowly dissolve and release drugs to the skin. The mechanical weakness of water-soluble materials leads to incomplete insertion of MNs and the extra time needed for dissolution. By designing the special separation structure, RSMNs are enabled to efficiently deliver drugs within minutes. In this study, to maximize the delivery efficiency and utilization of drugs loaded in RSMNs, detailed studies on structure improvements were conducted. By adjusting the length of the solid PLA MNs, the overlaps and the dissolving MNs, the optimal structure of RSMNs was determined with the capability to deliver over 95% drugs within 30 s. In transdermal delivery of insulin to diabetic mice, the optimized RSMNs successfully achieved a considerable delivery efficiency and treatment effect, no less than the injection therapy. From these results, the optimized RSMNs were shown to be a useful and reliable tools for transdermal drug delivery.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (51473017, 51673019), the Fundamental Research Funds for the Central Universities (buctr201406), the Innovation and Promotion Project of Beijing University of Chemical Technology, and the long-term subsidy mechanism from the Ministry of Finance and the Ministry of Education of PRC.

References