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Efficacy and safety of micro/nanostructured polymeric coatings for drug eluting stents

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Objectives Efficacy of a polymeric coating for drug eluting stents (DES) depends on its safety and structural properties. Today, it is well known that factors such as surface texture, morphology and drug release kinetics are among the most critical factors that determines the ultimate destiny and success of DES. Therefore, the ability to design and control these critical properties guarantee the success of DES in the body.
Methods In this study, two different micro/nanostructured coatings was prepared using poly(lactic acid) and dexamethasone by electrohydrodynamic atomization (EHDA) and spinning as coating methods. To analyze structural properties of coatings different techniques was used including: X-ray diffraction, scanning electron microscopy and confocal microscopy. Platelet, neutrophil and peripheral blood mononuclear cell adhesion was studied to evaluate safety of coatings. Then, antibacterial properties of coatings were considered. Finally, drug release profile was evaluated for 15 days.
Results The results showed that suitable surface properties of micro/nanostructured coatings led to very low platelet, neutrophil, PBMN on the surfaces. Micro/nanostructured coatings showed two drug release kinetics that are applicable for different drug delivery systems.
Conclusion Based on the results, EHDA method have great potential as a coating method for DES.
Keywords drug eluting stents, surface micro/nanotopography, drug release kinetics, electrospraying

Introduction

Nowadays, polymers is extensively used as coating on medical implants.[1] In addition to polymer properties, coating technique has remarkable effects on its surface and bulk properties. There are different implant coating techniques such as: Spin coatings, dip coating,[2] plasma spraying[3] and electrohydrodynamic atomization (EHDA).[4,5] However, there are currently few techniques that can produce polymeric coating with desired surface properties on medical devices.

Electrohydrodynamic atomization is a technique that has recently attracted much attention for biomedical application. EHDA have been used for several decades in different field of industry such as ink-jet printers and agriculture treatment. However, many new applications have been investigated. Examples of biomedical applications include: Mass spectrometry, nanoparticles production for pharmaceutical application and targeted drug delivery.[4,6,7] This is because of simplicity, easy controllability, low cost and large scale production potential. It is possible to provide homogenous coating on both side of highly complex and fine structures like drug eluting stents by EHDA technique. In an electrospray set up a syringe pump push out a solution to get out of a capillary nozzle that connected to high voltage power supply. Applied voltage produces high density of charge on exiting liquid droplets. This droplets are unstable and break up into very fine charged droplet that move toward a grounded or opposite charged collector. The collector should be conductive to electrospray can be continued. This is a unique advantage for coating implants with conductive metallic substrate like stents and increase coating efficiency and reduce solution waste. This is due to the deposition of sprayed droplets preferably occurring on metallic substrate rather than deposition onto any parts of the opposite surface.[5,6]

In this study, EHDA and spinning technique was used to create micro/nanostructured polymeric coatings on stainless steel plates. Poly(lactic acid) (PLA) with dexamethasone was used for coating. Then, the effects of resultant micro/nanostructures on coating properties coating was studied. Furthermore, safety and drug release kinetics of prepared coatings were evaluated.

Materials and Methods

Materials

Poly(lactic acid) (M_w = 260 kDa) and DAPI were obtained from Sigma. Dichloromethane (DCM), Tetrahydrofuran (THF) were purchased from Merck (Germany). All cell culture materials were prepared from Gibco Company (Ireland). Ficcole opaque and red blood cell (RBC) lysis buffer was from Biosera.

Coating preparation

For polymer-drug coating, a 1% w/v solution of PLA and DEX with a ratio of 85/15 was used in THF and DCM (50:50 v/v). EHDA instrument (Fnm Co., Tehran, Iran) was used for coating. To gain a uniform coating, samples were placed on rotating collector. EHDA condition was as follows: Applied voltage 7 V, flow rate 2 ml/h, tip to collector distance 7 cm and

collector speed 15 rpm. Spin-coating condition was done using the same EHDA instrument with the following parameters: Flow rate 8 ml/h, tip to collector distance 1 cm and rotator speed 15 rpm. Different groups shown in paper are as follows: EHDA–PLA–DEX, Spin–PLA–DEX.

X-ray diffraction

X-ray diffraction (XRD) were used for analysis of coating structures. XRD was performed using a STOE-STADV diffractometer (Darmstadt, Germany) with Cu Kα radiation and λ = 1.54060 Å. The scans were recorded at 2θ from 1° to 20°.

Coating morphology

The morphology of coatings were investigated using Philips XL30 scanning electron microscope (SEM, The Netherlands). A thin nanometric layer of Au (10 nm thickness) sputter was coated on samples before scanning.

Coating topography

The topography of samples was considered by confocal microscope and μsoft Premium software (NanoFocus, Germany). Root mean square roughness (S_a) of surface was reported as average surface roughness.

Wettability of coatings

Water contact angle measurement was performed to determine wettability of surfaces using goniometer (OCA 15 plus, USA). Thus, 4 μl droplets of water were placed on the surface and their images were captured. For each sample water contact angle of three different area on the surface were calculated and reported.

Platelet adhesion on the coatings

Scanning electron microscopy was used to study platelet adhesion on coatings. Anticoagulated human blood was obtained from healthy volunteers and centrifuged at 250 g at 25°C for 15 min to separate platelet rich plasma (PRP) from blood. Cell blood counter instrument was used to gain number of platelet. About 1 ml of PRP (containing 1,50,000 cells) was added to samples and incubated (2 h at 37°C). Then, samples were washed using phosphate buffered saline. For SEM, coatings were fixed with glutaraldehyde for 60 min and dehydrated with ethanol series (30–100%) and observed by SEM. Since collagen have specific domain for platelet adhesion, EHDA coated collagen was used as positive control.

Peripheral blood mononuclear cell and neutrophil adhesion on the coatings

Peripheral blood mononuclear cell (PBMC) and neutrophil adhesion were studied by DAPI staining. PMNC and neutrophil was separated from human blood by Ficoll-Paque. Centrifugation was performed for 30 min at 150 g. Then, buffy coat layer which contain PMNC was removed and the remaining RBCs were omitted using RBC lysis buffer. About 1 ml of solution containing of 1,50,000 PBMC was added to coatings in a 24-well plate and incubated (3 h, 37°). Finally, DAPI staining was performed using following protocols: Fixation for 30 min with paraformaldehyde (4%), staining with DAPI for 1 min and then washing with PBS. Finally, 10 images were captured by fluorescence microscope for each coating and cell counting were performed using ImageJ software. For neutrophil adhesion, the lower phase after centrifugation that contain RBCs and neutrophils was incubated with RBC lysis buffer and neutrophil were separated. All the other stages was similar to PBMC adhesion studies.

Antibacterial potential of the coatings

Agar test of samples against *Escherichia coli* ATCC25922 was used to evaluate bactericidal potential of samples based on 121CLSI protocol and using 2×10^8 CFU/ml of bacteria.

Dexamethasone release

For drug release studies, first a series of standards of dexamethasone was prepared (0–50 μg/ml) and a standard curve was drawn (maximum absorption wavelength of dexamethasone is 242 nm). Samples were placed separately in capped tubes containing 3 ml phosphate buffered saline. Then, samples were incubated at 37°C for 15 days. At determined time periods, 500 μl of solution was collected and replaced by 500 μl of fresh phosphate buffered saline. Then, dexamethasone absorbance in collected solutions was measured at 242 nm using spectrophotometry.

Statistical analysis

Data were reported as Mean ± SEM. Result analysis was done using *t*-tests. Significance of difference between samples was compared at *P*-value < 0.05.

Results

Fig. 1 shows X-ray diffractogram of samples. PLA have two sharp peaks at 2θ about 17° and 19°. These peaks are seen in Spin–PLA–DEX diffractogram. Thus, spin coating has no significant influence on PLA crystallinity. However, these peaks was not observed in EHDA–PLA–DEX coating. Therefore, EHDA samples had lower degree of crystallinity. DEX have a strongest crystallographic peak at 2θ about 13.54° that was obviously observed in Spin–PLA–DEX, but it was not seen in EHDA–PLA–DEX diffractogram. Thus, DEX mainly preserved its crystalline form in Spin–PLA–DEX.

Scanning electron microscopic images of coating is observed in Fig. 2a and b. EHDA coatings had microbead-and-nanofiber morphology, but, spin coatings had micro/nanoporous morphology.

Three-dimensional images of coating is shown in Fig. 3. The values for surface roughness (S_a) is shown in Fig. 4. EHDA led to surface with higher significantly higher roughness compared with spin coating method (1.734 ± 0.09 μm versus 0.908 ± 0.01 μm). Fig. 5 showed the results of wettability measurements. Water contact angle for ES–PLA–DEX and Spin–PLA–DEX was 130° and °96, respectively.

Scanning electron microscope images of platelet adhesion on coatings is observed in Fig. 6. There were many activated and aggregated platelets on collagen as positive control. While, very few number of platelets attached on coating surfaces is in non-activated form.

Furthermore, very low number of PBMC was observed on the surfaces of both samples that may be related to above-mentioned factors for platelet adhesion. Since neutrophil compose the main part of white blood cells and they are components of innate immunity system that immediately respond to foreign material, there was rather higher number of these cells on the coating surface (Fig. 7a–d). Fig. 8 shows the

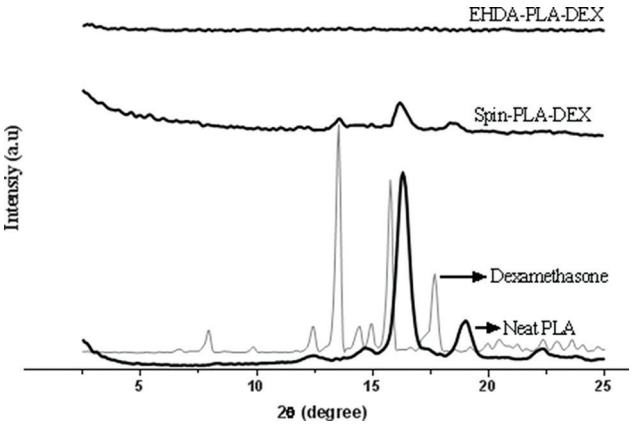

Fig. 1 **X-ray diffractograms of neat PLA, dexamethasone, Spin–PLA–DEX and EHDA–PLA–DEX.**

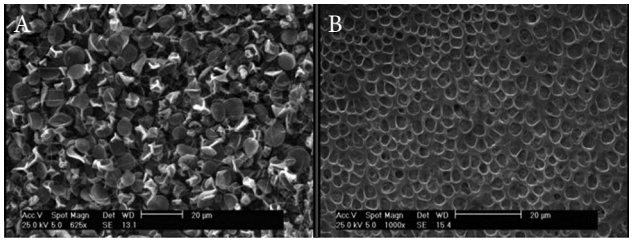

Fig. 2 **SEM images of the morphology of coating that show microbead-and-nanofiber structure of EHDA (a) and micro/nanoporous (b) structure of spin coatings.**

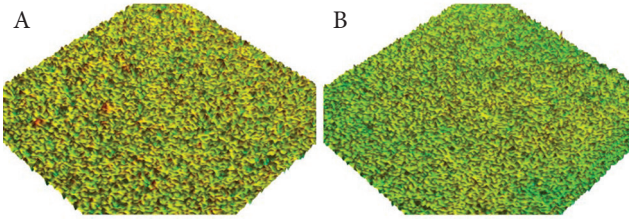

Fig. 3 **Three-dimensional images of coating surface using confocal microscopy.**

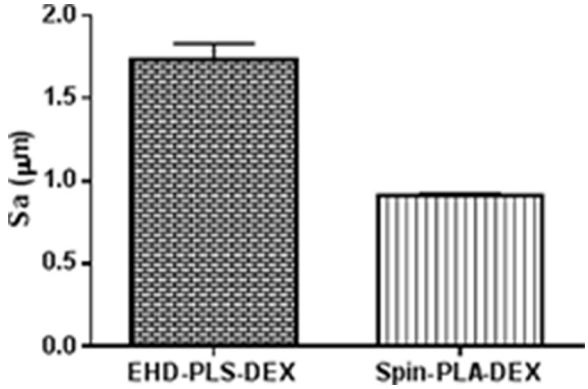

Fig. 4 **Root mean square roughness (Ra) of samples.**

numerical data of PBMC and neutrophil adhesion acquired by ImageJ analysis software.

The results of bactericidal effects of the coatings is observed in Fig. 9. The results of agar test showed that both samples had no bactericidal properties. This is related to lack of any antibacterial agents in the coatings.[8–10]

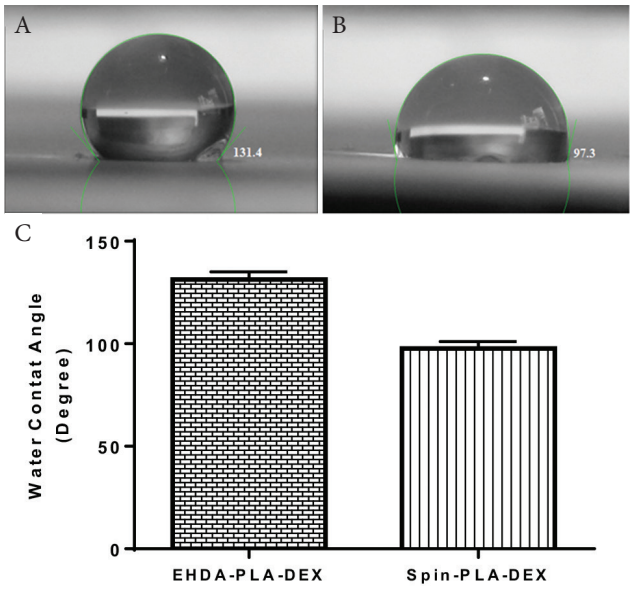

Fig. 5 **The images (a and b) and numerical values (c) of water contact angles of samples.**

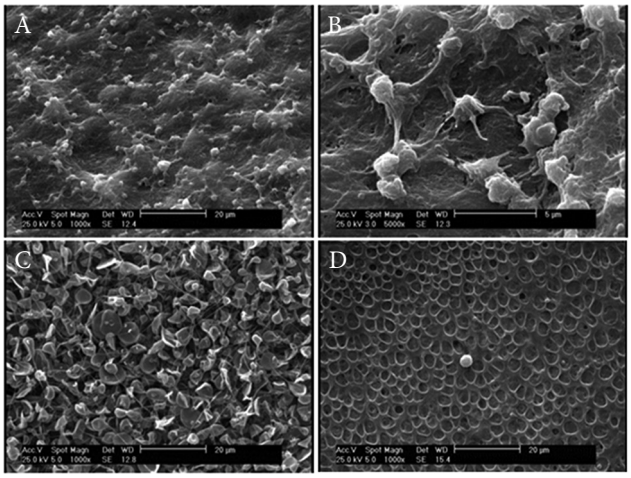

Fig. 6 **SEM images of platelet adhesion and activation on EHDA-collagen (a and b), EHDA–PLA–DEX(c) and Spin-PLA (d) samples.**

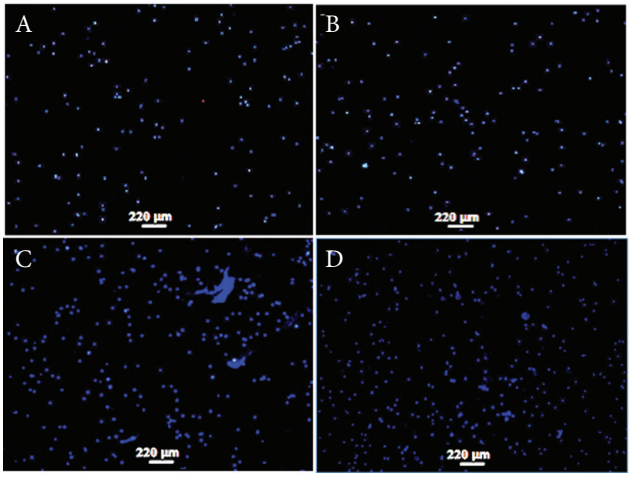

Fig. 7 **Fluorescence microscope images of PBMC and neutrophil adhesion on EHDA (a and c) and spin coatings (b and d), respectively.**

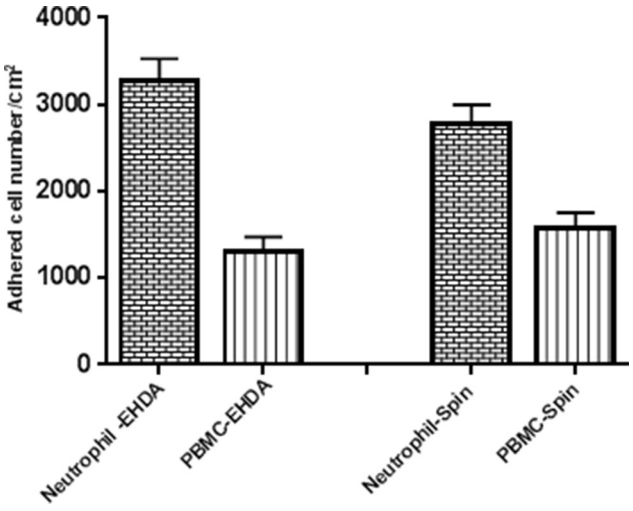

Fig. 8 **Numerical data of PBMC and neutrophil adhesion on the surface analyzed by Image J software.**

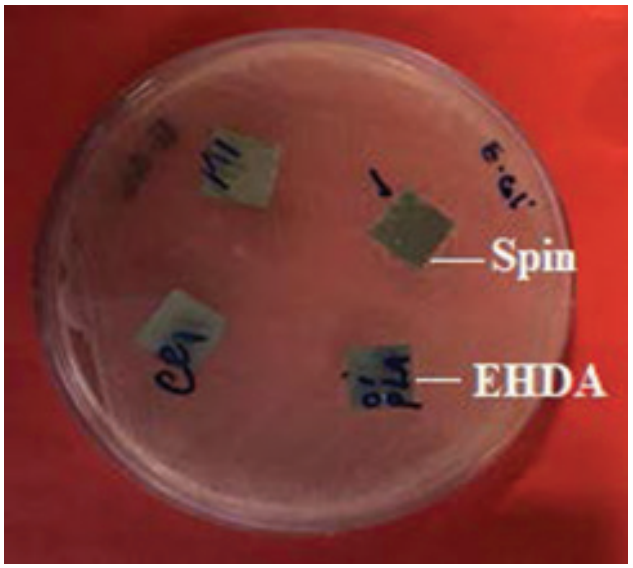

Fig. 9 **Agar test of bactericidal effects of coatings.**

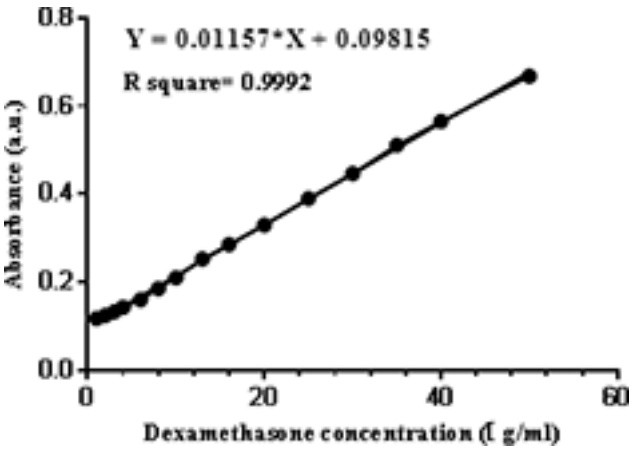

Fig. 10 **Standard curve of dexamethasone in the range of 0–50 µg/ml**

Dexamethasone release

The standard curve for dexamethasone is observed in Fig. 10. A linear relation with correlation coefficient of 0.999 was obtained for dexamethasone absorption in the range of 0–50 µg/ml. The release profile of dexamethasone from coatings is observed in Fig. 11. EHDA–PLA–DEX had a triphasic pattern of DEX release. The initial burst release of dexamethasone was 22% during first 6 h of study. Then, a second phase with nearly constant rate (lasted to 7 days) and third phase that had lower release rate compared to second phase (until to 15 days). The cumulative release was near 64% after 15 days for these samples.

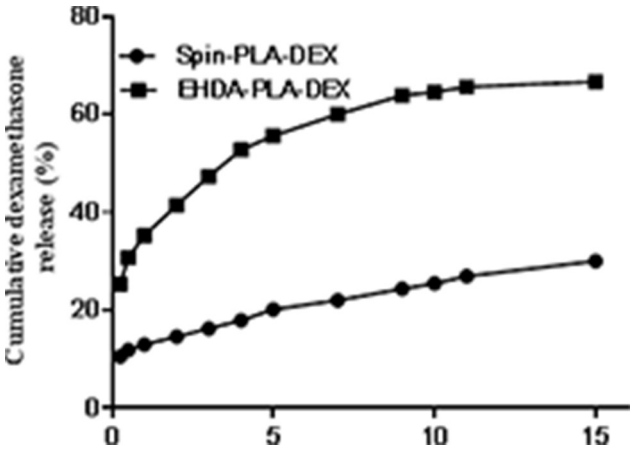

Fig. 11 **Dexamethasone release from different micro/nanotextured coatings.**

Spin–PLA–DEX coatings showed bi-phasic release pattern with 10% release after 6 h and a near linear release profile until the end of study. Where, only 30% DEX was released after 15 days.

Discussion

Polymer crystallinity is degree of crystalline regions in polymer structure and is related to alignment of polymer molecules. In this study, the results showed that EHDA cause lower degree of crystallinity for both PLA and DEX. These observation is due to rapid evaporation of solution in EHDA process due to low flow rate of solution, high distance between tip and nozzle and electrostatic repulsion. Thus, both polymer and drug does not have sufficient time to crystalize.[11] By controlling the determining factors of EHDA such as type of solvent, concentration and flow rates of solution, different product may be obtained including fibers,[12] particles[7] and their composites[6,13] in the range of micrometer to nanometer. In this study, we used condition to create a microbead-and-nanofiber morphology. Surface wettability is depended on both surface chemistry and micro/nanotexture. EHDA led to surface with significantly higher hydrophobicity. This is mainly related to higher roughness of micro/nanotextured coating of EHDA samples because of similar surface chemistry on both surfaces.[14,15] Results of platelet adhesion study showed very low number of un-activated platelets on coated surfaces compared with control collagen. Platelet adhesion depends on different coating properties. Previously, it has been shown that micro/nanotextured structures reduce protein and platelet adhesion on the surface.[16] Thus, hemocompatible nature of PLA, specific micro/nanotexture of samples and highly hydrophobic nature of coating possibly are the main factors that cause low platelet adhesion on the surface.[17–19] The results of drug release studies showed that EHDA coatings had triphasic release pattern with

a high burst effect. However, spin coated samples had a biphasic pattern with very low burst release. Drug delivery systems such as Spin–PLA–DEX are favorable for sustained drug release. While delivery systems such as EHDA–PLA–DEX are suitable for applications like drug eluting stents. Because of physical damage to arterial wall during stent implantation, there is need to rather a high amount of anti-inflammatory drug during first hours and days after implantation.[20,21] For example, endeavor is among the most efficient drug eluting stents which release about 80% of its anti-inflammatory during first 10 days after implantation.[22] The burst release from samples is mainly related to drug that is on the surface or near the surface.[23,24] The second phase is probably related to diffusion of dexamethasone from coating matrix and also very little degradation of PLA. The third phase is due to diffusion and further PLA degradation and erosion.[25] Various parameters have an important role in drug release behavior such as type of polymer and drug, morphology of coating and degree of crystallinity of polymer, molecular weight and hydrophobicity of polymer.[26,27] The polymer and drug was similar for both micro/nanotextured coatings. But, crystallinity of coating was significantly different. Higher amorphous regions in the EHDA–PLA–DEX coatings led to higher drug release due to higher penetration of water into amorphous regions.[28] But, higher crystallinity in Spin–PLA–DEX samples limits mobility of polymer chains that decrease DEX release.[29] Also, higher roughness of EHDA samples lead to higher surface contact area for these coating that in turn increase drug release rate specially from nanofibrous parts of EHDA–PLA–DEX samples.[30,31]

Conclusion

In this study, EHDA and spinning technique were used to produce two different micro/nanotextured coatings on stainless steel plates. The impact of resultant micro/nanostructures on safety and efficacy of coating for drug eluting stents application showed higher efficacy and safety profiles of EHDA coating due to their specific microbead-and-nanofiber structures.

Acknowledgment

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Conflict of Interest

None.

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