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# Electrospun zwitterionic nanofibers with in situ decelerated epithelialization property for non-adherent and easy removable wound dressing application

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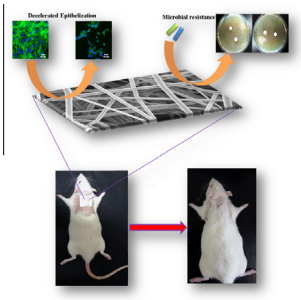
## HIGHLIGHTS

- Non cell adherent wound dressing membranes.
- Easy removable, no-pain wound dressing bandages.
- Blood inert wound dressing membrane, suitable for large areas of chronic wounds.
- Resist microbial biofilm formation and hence provides minimum chance of infection.
- Minimum cosmetic scar due to less cell adsorption on wound dressing membrane.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Wound care management is a serious issue among the medical practitioners due to its varying complexity and various materials were tested for fast relief and easy removal. In this regard zwitterionic polymer based wound dressing membranes are the key point of attraction. Here we prepared a novel zwitterionic poly (carboxybetaine-co-methyl methacrylate) (CBMA) copolymer based nanomembranes using the electrospinning technique for the wound dressing application. The study takes advantage of the outstanding chemical properties of zwitterionic CBMA and the morphological efficiency of nanomembranes. The cell attachment studies proved the cell inert nature of thus prepared membranes. Such non cell adherent wound dressing membranes can be applied as the easy removable, no-pain wound dressing bandages. Our results clearly showed that the excellent blood-inert nature can be achieved by the CBMA nanofiber membranes. Therefore, there will be less chance of attaching blood clot with the wound dressing membrane and is extremely significant for the care of patients with large areas of chronic wounds. Additionally the in vivo results showed the formation of new tissues within two weeks, evidence of a complete wound healing material. So our CBMA membrane can be successfully used as a perfect wound dressing material with minimum cosmetic scar.



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## 1. Introduction

The skin is considered to be the largest organ of the body, and is serves many different functions. Therefore, wound care management is a serious healthcare issue. Wound dressings are primarily responsible for managing the most types of open wounds [1]. The immediate care of skin wounds is important to prevent microbial infections as well as trans-epidermal water loss that may cause acceleration in the wound regeneration [2]. Therefore, the restoration of the skin barrier is of crucial importance for the treatment of injuries [3]. Wound dressing materials made via electrospinning technique have been increasingly studied due to their superior properties, and the electrospun nanofibers have been appropriate for use as perfect wound dressing materials as a result of their useful properties, including flexible pore-size distribution, high porosity to provide oxygen permeability, and high surface-to-volume ratio [4–6]. A variety of materials have been electrospun to control the microenvironment of the wound surface as well as to stimulate the healing process, and currently nonadherent, wound dressing materials have been preferred in the sense that they can be easily removed without trauma, possesses antimicrobial properties and promote wound healing with minimal scarring [7–10].

Natural biopolymers, such as chitosan [11,12], alginate [13], cellulose acetate [14] and, hyaluronic acid [15] have been extensively studied for their potential role as materials for wound dressings. The use of such biopolymers is sometimes limited due to their dissimilarities from batch to batch of raw materials and the possibility of the transmission of an infection due to their bioactive nature. Many synthetic polymers, such as polyurethane [10,14], poly (ε-caprolactone), and poly (L-lactide) [16], have also been used as wound dressings. The mechanical properties, desirable cytocompatibility and low cost of the synthetic polymers affirm that these are perfect materials for use in wound dressing applications [17]. However, most polymeric materials lack resistance to the nonspecific adsorption of proteins. This can result in nonspecific cell attachment and in bacterial adhesion, which in turn would cause bacterial infections and pain upon removing the wound dressing material as a result of the cell growth over the material [18].

Recent studies have shown that a zwitterionic copolymer composed of carboxybetaine methcrylate monomer exhibits excellent blood compatibility, including the suppression of platelet adhesion and complement activation on the surface of the polymer [19]. They are also known to be resistant to cell adhesion, both in vitro and in vivo [20]. The zwitterionic carboxybetaine polymers have also been reported to possess antibacterial properties and resistance to the formation of bacterial films [21,22]. Their desirable properties, such as a strong resistance to protein adsorption, cell attachment, and resistance to bacterial growth, have made carboxybetaine methcrylate polymers promising materials for use as nonadherent wound dressing materials [23]. Such membranes will not damage healing tissue and can be easily applied and removed, causing no pain [18]. Furthermore, electrospinning is an easy, efficient method that can be used to prepare such nanofibrous membranes. The electrospun nanofibers have been found to be very effective when used as wound dressing materials mainly due to their architectural superiority [24].

We synthesized poly (carboxybetaine-co-methyl methacrylate) copolymer (denoted as CBMA throughout this paper). The application of the methylmethacrylate based material as a wound dressing has already been extensively reported [25]. In this work, a fibrous membrane was electrospun from as-prepared CBMA polymer for use as a wound dressing. The main objective of this study is to develop an electrospun nanofibrous membrane from CBMA in order to study its properties when intended for use as active nonadherent wound dressings. The cell and platelet adhesion behavior and the in vivo wound healing efficiency of the zwitterionic CBMA nanofibrous membrane were thus studied.

## 2. Materials and methods

### 2.1. Synthesis and characterization of the carboxybetaine monomer [CBAA-5-ester: (6-carboxypentyl)-3-acrylamidopropyl dimethylammonium bromide, ethyl ester]

The carboxybetaine monomer was prepared and characterized following methods that were previously reported [26]. N-(3-Dimethylaminopropyl) acrylamide (50 mmol), ethyl 6-bromohexanoate (55 mmol), and acetonitrile (25 mL) were added into a 100-mL round-bottom flask. The mixture was stirred under a nitrogen atmosphere for five days at 45 °C. The solvent was removed on a rotary evaporator, and the product was then precipitated and washed with anhydrous ether. In the next step, 100 mg/mL of monomers were dissolved into aqueous solutions with 1 M NaOH concentrations. After 24 h of hydrolysis, the solution was neutralized with a dilute HCl solution and water was then removed by vacuum. $^1$H-NMR (400 MHz, D2O): 2.00 (*m*, 4H, C—CH2—C), 2.47 (*t*, 2H, CH2—C═O), 3.1 (*s*, 6H, N+(CH3) 2, 3.3–3.4 (6H, CH—N and CH2—N + —CH2), 5.75 (*m*, 1H, CH═C—CON-trans), 6.19 (*m*, 1H, CH═C—CON-cis), 6.26 (*m*, 1H,═CH—CON—).

### 2.2. Synthesis of poly (carboxybetaine -co-methyl methacrylate) copolymer abbreviated as CBMA

An appropriate amount of two monomers, MMA (47 mmol) and carboxybetaine monomer (4.7 mmol), were added to a 50-mL round bottomed flask that contained 30 mL of dimethylformamide. The solution was bubbled with nitrogen for 10 min, azobisisobutyronitrile was then added to the flask, and the solution was heated up to 70 °C and was stirred overnight. Next, the solution was added drop-wise to 400 mL of diethyl ether while stirring to precipitate the synthesized copolymer. The purified copolymer was dried overnight in a vacuum oven at room temperature. The final products present a white to light yellow color and had a yield of 68%. The molar ratio of carboxybetaine to MMA in the obtained copolymer was estimated to be 9.8% by $^1$H-NMR analysis (Fig. 1 supporting information) [27,28] by taking the integral of a peak at 3.4 ppm belongs to the six protons present in the two methyl groups attached to the quaternary amine ($(CH_3)_2N^+$) of carboxybetaine pendant group, and a peak at 3.7 ppm belongs to the three protons present in the terminal methyl group of the MMA pendant group. It should be mention that deuterated chloroform was used to dissolve copolymer and which results in the sharp peak at 7.24 ppm. The materials were also tested chromatographically, but due to the poor solubility of the materials, it was not possible to provide exact values, even with the addition of salts such as LiCl [29]. The carbobetaine repeat unit of the copolymer is hydrophilic, and makes the polymer insoluble in the relatively hydrophobic solvents [29]. However, since the polymer solution is spinable it can be said the molar masses are high enough for the electrospinning process.

### 2.3. Fabrication of the CBMA nanofibers

Copolymer solutions were prepared by dispersing the polymer at concentration of 20 wt% in dimethyl formamide (DMF). The obtained solutions were placed in a plastic syringe tube and were fed through a metal capillary (nozzle) with a diameter di = 0.21 mm (21 G) attached to a 1-D robot system that moves laterally and is controlled by the LabVIEW 9.0 software program