ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Synthesis and Characterizations of Chitosan/EDTA/PVA Nanofiber for Antimicrobial Applications

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Abstract

The aim behind this study was to fabricate thermally crosslinked Chitosan. (CS)/ Ethylenediaminetetraacetic acid (EDTA)/ Poly (vinyl alcohol) (PVA) and pure chitosan as nano fiber mats. The CS/EDTA/PVA nanofibers and/or pure chitosan were prepared using elctrospinning technique. The suitable cross linking ratio and cross linking times for CS/EDTA/PVA nanofiber were look into. The scanning electron microscopy and X-ray Diffraction was utilized to investigate influence of adding polymers to chitosan on the morphological and diameter of the nanofiber. The FTIR was carried out to evaluate the spectroscopic properties and functional groups which present in the compounds. The antimicrobioal test was examined by using Staphylococcus Aureus which is a spherical gram-positive bacterium and common test for antimicrobial applications. The result of antimicrobials test was made the CS/ESTA/PVA as nano fiber is viable materials for biomedical applications.

Keywords: *Antibacterial, Biopolymer, Nanostructure, Physical properties.*

Introduction

The growth of low price polymer thin films in many applications is based on conduction mechanism and electrical properties of used materials [1]. A lot of new techniques are used to fabricate polymers Films hoping to control material properties with low manufacturing cost. During the past decades, nano structured Materials were used because of its extensive applications. Synthesis techniques, characterization, and treatment of nano structured Materials are a novel field that grows well and fast [2].

Researches and developments in the field of nano structure are Emphasizes a modern scientific discovery polymer material with control the nano structure Properties and processed in bulk materials with technological functions and engineering properties [3]. So, they introduced new concepts of mechanical techniques and manufacturing methods. Many articles reached and manipulated (ID) nano-structures because if their Distinct physical and chemical properties.

Between the all one dimension nano structured polymer materials, the nano fibers has been individual consideration due to the good mechanical properties, manageability, long axial ratio and an interesting physical properties. The nano fibers are presented as an excellent support a wide range of diverse and potential applicated [4, 5]. Electrospinning is very simplest and at ease technique to control the morphological and diameter of fibers, [6]. The electrospun technique was used widely for soluble polymers or fusion alone. Nowadays, some additives such as particles or enzymes were added to Polymers to obtain the required properties [7].

Accordingly, the polymer as nano fibers was used in almost of biomedical applications such as using it in dental tissue engineering [8], drug delivery systems [9], enzyme Immobilisation [10], and/or wound healing [11]. Chitosan is one of the important biopolymer in biomedical application due to their anti-microbial using and anti-fungal

activity [12]. In general, chitosan is a natural non toxic can be derived from the deacetylation of chitin, which can expressed by ($C_6H_{11}O_4N$) formula [13]. Several articles showed that many model were indicated that the chitosan had a novel antimicrobial activity due to its cationic nature [14, 15]. They were expected that an electrostatic interaction occur between Positive charged of R-N $(CH3)_3^+$ sites and negatively charged of microbial cell, which is responsible for cellular lysis and assumed as the main antimicrobial mechanism [16].

Also, they suggested, when the charge density of polymers become higher, antimicrobial activity will be improved [17]. Electrospinning nanofiber is one of the best ways to reformation of chitosan from bulk to nano structure and uses it in antimicrobial test [18]. The main purpose of our report is synthesis chitosan (CS) as nano fiber with crosslink of Poly (vinyl alcohol) (PVA) and ethylenediaminetetraacetic acid (EDTA), test the structure & spectroscopic properties by SEM, XRD, FTIR, UV, and exam the antimicrobioal activities.

Material and Methods

Chitosan (grade of deacethylation 0.85, MW 110 kDa, Ethylene-diaminetetra-acetic acid (EDTA), and Poly vinyl-Alcohol (PVA) (grade of poly merization » 1600, grade of hydrolysis » 97.5 - 99.5 mol %) was purchasing from various suppliers. These materials were used without any further purification. All solvents and reagents were used without any additional purification. The morphology and diameter of the chitosan and/or CS/EDTA/PVA nano fiber were examined by using scanning-electron microscopy [SEM-Tescan VEGA 3 SB]. The crystallinity and crystal phase for CS and/or CS/EDTA/PVA were studied by, using [Rigaku X-ray-diffracto-meter XRD-6000-Shimadzu, .Japan). The physical propertion and chemical structure were characterized by using FTIR [FTIR 8400S, Shimadzu Japan] and UV- Vis (AA 6300 - Shimadzu -Japan]. The antimicrobial test was made in vitro.

Preparation of CS/EDTA/PVA Nanofibers

Chitosan with ratio 2% (w/v) and EDTA solution was prepared. It was dissolved in DW (Dis, Water) at a weight ratio 2: 1. The mixtures were prepared by stirring at 80 C° for 3h. PVA solution with 12% (w/v) was dissolved in DW (Dis, Water) at 80 C° and Followed by Stirring with heat at 70 for 2 h. The CS- EDTA solution was added to PVA solution at a weight ratio of 30:70. The electro-spinning process was conducted; at room temperature (27C°) and clamminess was a well nigh 33%. The electro-spun nano fibers were deposited on the Exterior of Disk and Aluminum sheet. The applied voltage was 24 kV and tip-to-collector distance (TCD) was about 15 cm and feeding rate as 0.4 ml/h. The collected CS/EDTA/PVA nano fiber mats were collected from Aluminum sheet. The preparing EDTA/ CS /PVA nano fibers were thermally cross-linked by placing it in an oven at 90 C° for 3 h.

Antibacterial Examined

The antimicrobial activity of the Chitosan, CS/EDTA, and/or CS/EDTA/PVA nano fibers was examined against St,Aureus ATCC 6538P as mentioned in the Natthan at el procedure. (19). For the Minimum Inhibitory -concentration (MIC) test, S. aureus was Implanted in Tryptone soy-broth (TSB) and put in the shaking or vibration incubator at 38C° and110 rpm for 24 h. After that, the bacterial suspension was alleviated until the Bacterial-concentration was around 1×10^6 cfu/ml, then it was pipette or absorbent in a well plate at a concentration of 1 ml.

Nanofiber mats were placed in wells containing bacterial suspension and incubated at 38C° for 24 h and The MIC was observing after 24 h of Incubation. For determining the minimum bactericidal concentration (MBC), the Blend of wells without growth (100 µl) was spread onto agar plates.

The minimum bactericidal concentration (MBC) was watched after approximately one day of incubation at 38C° on the agar plates. After that the antibacterial properties of the CS, CS/EDTA, and/or EDTA/CS/ PVA nano fiber mats were evaluated by the Agar Diffusion-test (ADT). A three different pieces from nano fiber mats (1 cm in diameter) was place, on the Surface of S. aureus bacterial solutions in agar plate, and were made stabbed uniformly. Two days incubation at 38C° later, the inhibition zone and inhibition zone circuit count area were identified.

Results and Discussion

CS/Edta/PVA Nanofibers

Electro-spinning of CS/EDTA/PVA solution was carried out. The using of electro-spinning technique involves applying a high voltage to charge the surface of a water solution. A polymer which stimulates the expulsion of a liquid jet through the spindle .Because of the bending instability, the jet is extended several times to form micro fibers [20]. One electrode was set in the syringe of spinning-solution and another one was connected to a collector. Electric field is subjected to the end of a tube that contains the polymer solution handle by its surface pull. This will create an induced charge on the surface of the liquid. Distribution charge baffle causes a force opposite to the surface pull from solution. When the electric -field start to increase, cone-shape will be formed and made hemispherical shape from the fluid at the end tip of the capillary tube [21] and a charged polymer fiber will be left at the tip. These charges will be implanted randomly on a grounded collecting metal screen [22]. The nano fiber mats which get it from used CS/EDTA/PVA Gelatinous liquid were shown in Fig1.

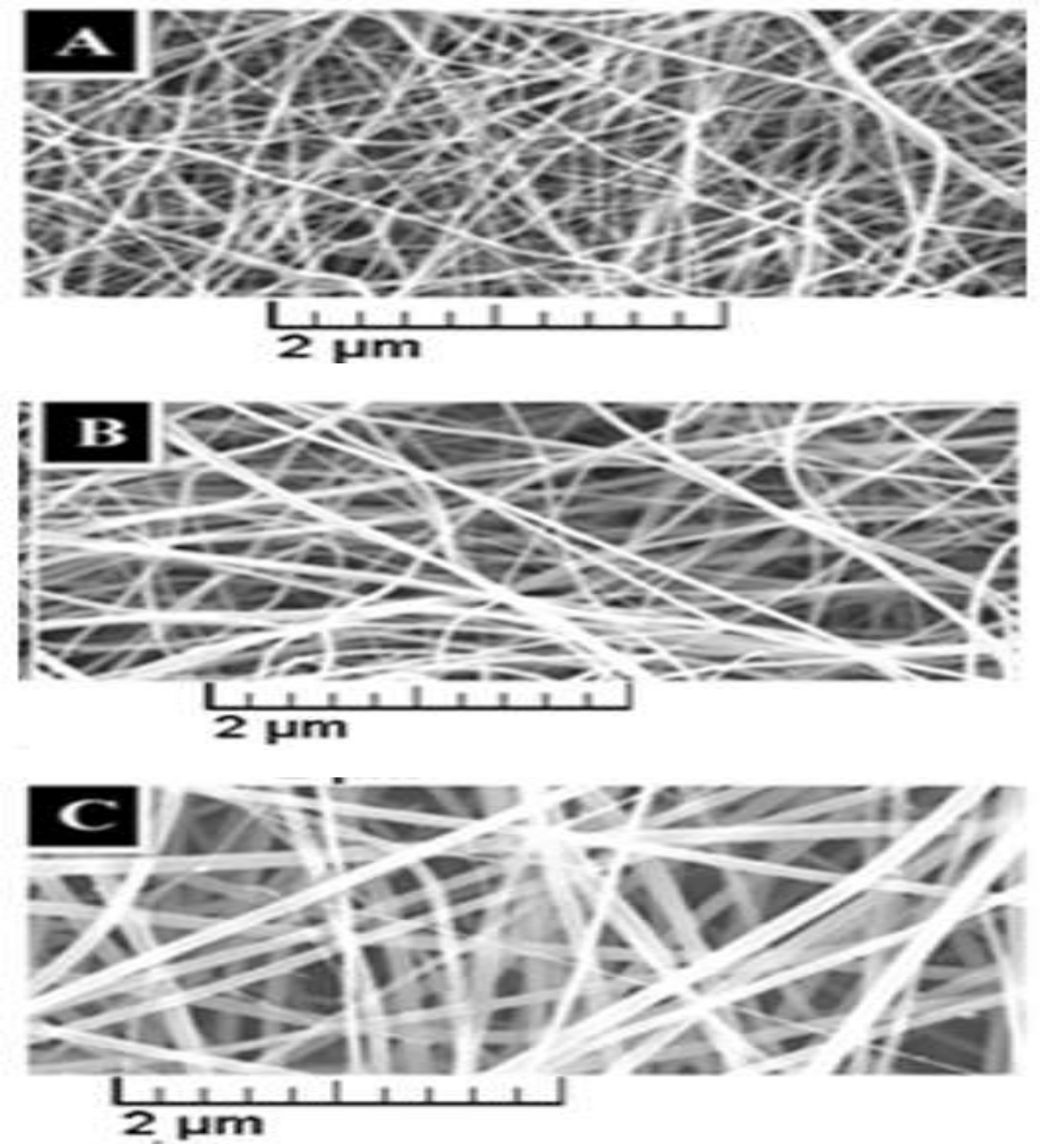

Fig.1: SEM images of a) pure chitosan, b) CS/EDTA, and c) CS/EDTA/PVA

The smooth bead-free uniform-fibers were observed and well morphology nanofibers were obtained. The average diameter-zone of the obtained nano fiber was 157.76±42 nm.

The X-ray diffraction was measured to examine the crystallinity of CS/EDTA/PVA nano fiber mats. Fig. 2 shows the XRD patterned of raw chitosan, chitosan/EDTA, and chitosan/EDTA/PVA.

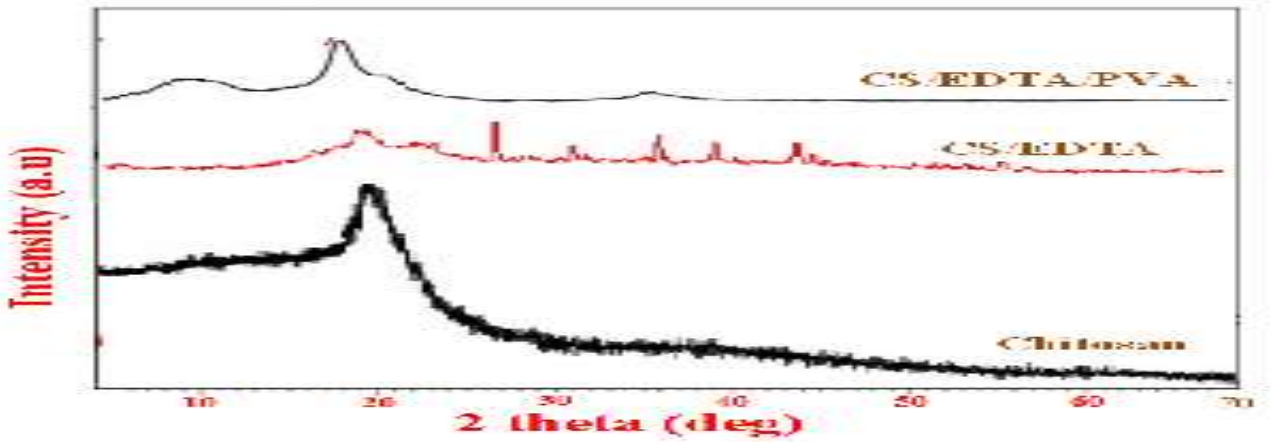

Fig.2 XRD patterned of pure chitosan, CS/EDTA, and CS/EDTA/PVA

The effects of the EDTA and/or EDTA/PVA as crosslinking on the crystallinity of CS nano fiber are very clear. In addition, as the EDTA and/or PVA mixed with chitosan, there are many interference peaks. The X-ray diffraction peaks for chitosan nano fiber were found a broad, so it matched with that of amorphous polymer [23]. Chitosan showed crystalline broad peaks at 2θ of 19.8˚ for (102). When chitosan mixed with EDTA, the crystalline peaks were observed at 2θ of 19.8˚, 23.9˚, 27.2˚, 31.1˚, 36.2˚, 38.8˚, and 43.6˚. When the PVA was added to CS/EDTA with high ratio (30/70), the intensity of diffracted peaks became very low, and The peaks were crystalline observed at 2θ of 9.1˚, 17.9˚, 20.1˚, and 35.7˚ and some peaks were faded out. The amorphous diffraction peak was observed at 2θ of 19.8˚ and indicative as the blending of PVA/chitosan. All the diffraction summit is indexed to the spinel-structure (JCPDS no. 00-003-0863) and suggested that chitosan / PVA fibers be lower crystallized than chitosan fibers. The reduction of crystallization of it was probably due to the interaction of the hydrogen bond among molecules of PVA and chitosan [24]. Fig. 3 shows FTIR spectrum of the electro-spun fibrous that was ready from pure chitosan, CS/EDTA, and/or CS/EDTA/PVA solutions.

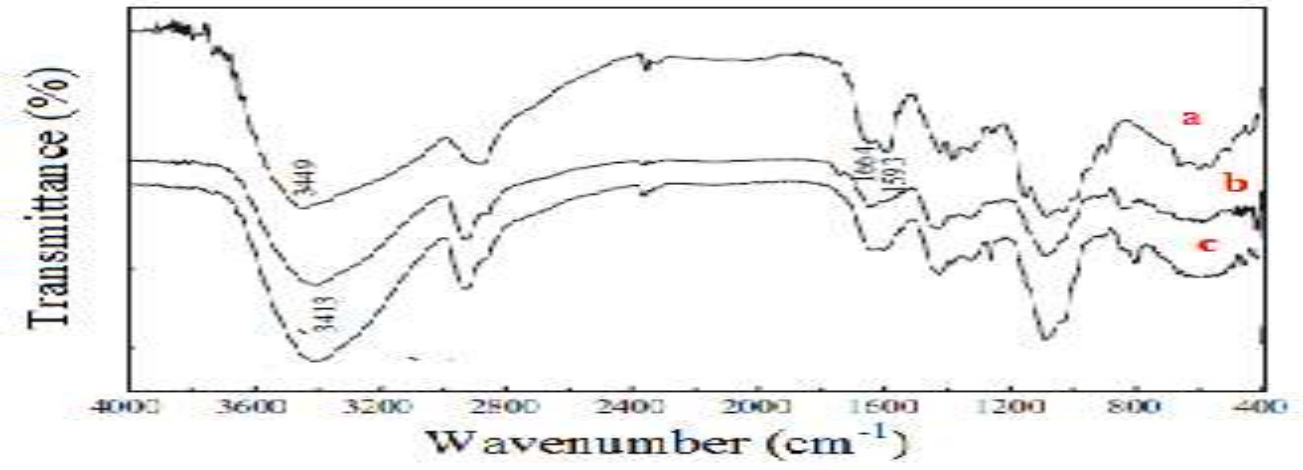

Fig.3 FTIR spectra of a) pure chitosan, b) CS/EDTA, and CS/EDTA/PVA nano fibers

FTIR spectrum of chitosan and Cs/EDTA is shown in Fig. 3-a & 3-b respectively, the peaks absorption for carbonyl stretching vibration in O=C–NHR groups, N–H bending shaking in amine-groups, and N-H stretching vibrations were observed at 1664 cm^{-1}, 1593 cm^{-1}, and 3449 cm^{-1} respectively [25]. Fig. 3-c shows high stretching vibration in PVA hydroxyl groups at 3413 cm^{-1}. When EDTA and EDTA/PVA were adding to the chitosan, the assimilation peak at 3449 cm^{-1} was relocated at lower wavenumber. The absorption peak of amino groups at 1593 cm^{-1} was gradually vanish because the volume of

chitosan was decreased in electro-spun mats as well as the interaction between chitosan and PVA is macromolecules. It may be point out that hydrogen bonds among hydroxyl groups in both PVA and amino-groups or in chitosan could possibly take place. Therefore, adding PVA solution Can be moderate the interaction between chitosan macromolecules as well as improve the electrospinning ability of chitosan / PVA mats [26].

Antibacterial Activity

The inhibitory effects on the bacterial growth from chitosan first were evaluated by turbidity measurements. A lot of mechanisms for anti-microbial action of Chitosan have been assumed. There is as follows: (1) Chitosan can be scratched with tiny elements or essential nutrients, so it prevented growing of bacteria [27]. [2].

It can interact anion groups at the surface area of the cell and have complex compounds of electrolyte with the surface bacterial compounds [28]. So, it may form an impervious layer around the cell and preventing the transfer of the basic solids to the cell [29]. The Anti-bacterial ability of the nano fibers samples were examined in terms of the Inhibition zone created on agar around the paper discs against S. aureus as shown in Fig.4.

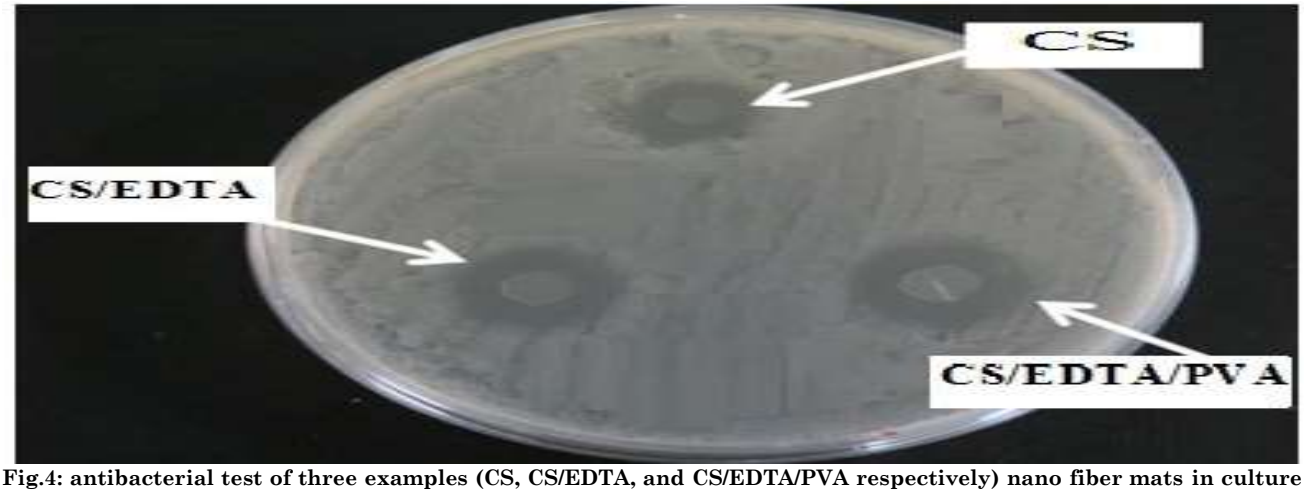

Fig.4: antibacterial test of three examples (CS, CS/EDTA, and CS/EDTA/PVA respectively) nano fiber mats in culture medium inoculated with Staphylococcus Aureus

The pure chitosan had a diameter inhibition zone around 1cm. The inhibition zone diameter (D) increased gradually for chitosan mixed with EDTA and PVA, and became1.4 & 1.7 cm respectively. So, we think the crosslink between chitosan and EDTA or CS/EDTA and PVA play essential roles to improve the antibacterial properties.

This results matched with changing in crystallinity of chitosan in XRD patterned and created the hydrogen bonds between amino groups or hydroxyl groups, and hydroxyl groups in PVA in chitosan. This suggested that macromolecules interaction between chitosan and PVA acted to improve the antimicrobial activities. In summary, chitosan, CS/EDTA, and CS/EDTA/PVA nano fiber have been characterized and synthesized, in the attendant study. The obtained result shows a uniform nano fiber with bead-free. The nano fibers obtained in this study have very small diameters with positive charges on the surface, which may improve their constancy in the presence of biological cations as well as improve for their anti-bacterial activities *In Vivo*.

This study shows that CS/EDTA or CS/EDTA/PVA nano fiber could inhibitor the growth of micro-organisms noteworthy and exhibit higher anti-bacterial activity than chitosan nano fiber alone. These biodegradable, biocompatible and antibacterial electro-spun nano fiber mats of CS/EDTA/PVA have promising potential for use as effective biomedical materials, and low cost membrane.

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