FUNCTIONALIZATION OF ELECTROSPUN NANOWEB USING ETHIOPIAN ENDEMIC PLANT

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EXTENDED ABSTRACT

Key words: Endemic plant, Ethiopia, nano-fiber, wound dressing, active extract, and characterization

The aim of our work is to develop a functionalized patch for wound dressing application using natural and endemic plant extract from Ethiopia.
Acokanthera schimperi (Apocynaceae) "Yemerz Enchet" is one an Ethiopian based plant in which its extract is used for treatment of chronic wounds by the traditional doctors.[1] Therefore, it has been selected to be used in this study. Different Hydroalcoholic extracts of medicinal plant have been tested and the more efficient one has been selected for the following studies.
As it is well known, electrospun nanofibers patches are an ideal candidate which satisfies the properties of efficient wound healing applications such as mimicking extracellular matrix structure. Moreover, an efficient bacterial barrier, an appropriate water vapor transmission rate and provision of adequate gaseous exchange is also provided by such kind of patches.[2]
The aim of this presentation is unrolled along the different steps that have been followed to functionalized the nano-filament patches with the selected plant "Yemerz Enchet". The challenge to incorporate "Yemerz Enchet" extract into the polymeric nanoweb is also discussed. The polymer that has been chosen, regarding the literature recommendation is the PCL. The production parameters have been selected and adapted from literature issues thank to different trials.
Uniform and bead free nanofibrous mats loaded with 1,2 and 3% extract were produced. The fabricated nanofibrous is characterized using SEM and FTIR in order to characterize the modification that occurs during the electro-spun process. FTIR analysis indicates that the chemical nature of extract and polymer polymer are not affected by the electrospinning process. The biologic test, that is currently running, will characterize the anti-bacterial properties of the selected extracts and of the final products.

1. INTRODUCTION

Traditional medicine plays a significant role in the healthcare of the majority of the people in developing countries, including Ethiopia, and medicinal plants provide valuable contribution to this practice. Skin infections (wound) are among major health problems in Ethiopian which can be treated with endemic traditional medicines. "Yemerz enchet" is one of the most common and effective endemic traditional medicine used for healing wounds applying directly its crude extract by practitioners in the country. [1] The crude extract could contain microorganisms, making them potential sources of infection and the difficulty to determine the dosage required

to heal the wound are the two most challenges of medical plants when directly applied on wounds. This leads inconveniency of the patients during treatment and requires long time of curing.
In recent years, researches on electrospun nano-material have been viewed with tremendous interest on biomedical applications due to their unique characteristics such as very high surface area-to-volume ratio, high porosity with very small pores size and small diameter which mimic the topology of extra cellular matrix presents in human body and therefore can serve as excellent carriers for therapeutic agents that are antibacterial or accelerate wound healing. [2][3]

2. MATERIALS AND METHODS

2.1. Materials and devices

As mentioned previously, PCL (Polycaprolactone) has been chosen based on literature review. The selected PCL presents a molecular weight Mn=80,000 and has been purchased from Sigma-Aldrich, (Germany) in pellet form. The literature review provides the information about the appropriate solvent to be used with such kind of polymer and extracts. Chloroform, methanol, hexane, and ethyl-acetate were obtained from Merck Co. (Germany). Electrospinning process was performed on Elmarco's needle-free Nanospider™ 1WS500U machine (Czech Republic).

2.2. Extract Preparation

Extraction of the active components of the herbal plant was done by maceration.This maceration is obtained thanks to 50gr of herbal plant in dried and powdered forms mixed with 500ml of aqueous solution of methanol (80%) taht were stirred for 24 hours at room temperature. The solution was subjected to evaporation under vacuum and freeze-drying after filtration through Whatman paper. The dried extract was fractionated again with an aqueous solution of Hexane: Water (2:1), Chloroform: Water (2:1), Ethyl acetate: Water (2:1) and Methanol: Water (2:1) to have different solvent extracts [4].
The analysis of the extracts with gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) gave evidences for the presence of different phytochemicals like Kaempferol, Chlorogenic acid, Rutin etc which are useful in anti-inflammatory, anti-microbial, and anti-oxidative of wound healing process.

2.3. Electrospining process

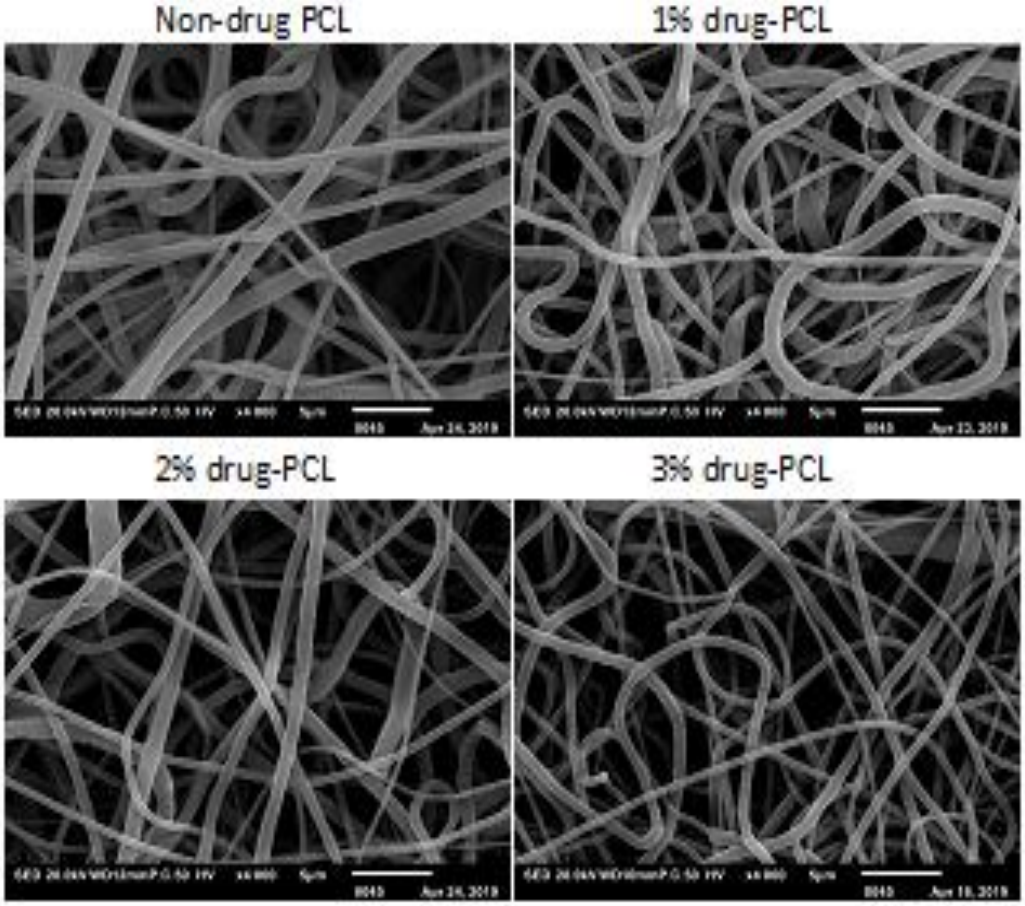
PCL solutions with the 13% (w/v) concentration were prepared by dissolving the polymer in a mixture of chloroform/methanol (3:1) as a solvent system and stirring for 24 hours at room temperature in three separate containers having a capacity to hold 40mL. After complete dissolution of the polymers, herbal extract of 1%, 2% and 3% of total polymer weight was added separately to the three bottles and the stirring process continued for 5 hours. One bottle remains without drug as referece sample for comparison.
To electrospun the prepared solutions, the following element and adjusteents have been chosen on the ElMarco machine : 0.6 metallic nozzle, 40kV, 150mm electrode to collector distance and 10min spinning time. The process was performed at relative humidity of 40% and 21^0C as température.

3. CHARACTERIZATION

3.1. Morphology of PCL-drug nanowebs

For morphological studies, scanning electron microscopy JEOL's JSM-IT100 was utilized. The samples were observed after gold sputter-coating. Then, the average diameter of about 50 nanofibers in each drug-loaded concentration and drug free image was calculated using Image J software. Figure 1 shows SEM morphology of the electrospun pure PCL and PCL-drug nanofibers.

Fig.1 Fiber morphologies of pure and drug loaded PCL electrospun fibers

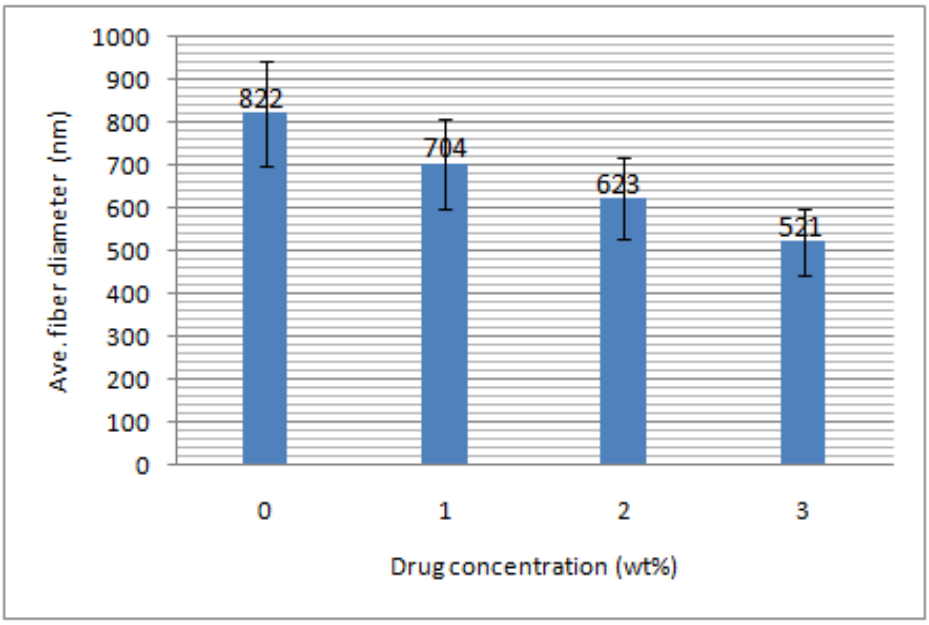


In all the produced nanofibers, the standard deviation in fiber diameter decreases as the concentration of the drug incorporated increases without any beaded morphology. This is clearly seen from fig 2 where the standard deviation for pure PCL is 177.5nm and 67.7nm for 3% drug incorporated in the PCL matrix.

From the SEM images, it is evident that the fiber diameter decreased to a large extent while incorporating the drug. The fiber diameters were calculated from the SEM micrographs and are shown in Fig. 2. Average fiber diameter of pure PCL membrane was 822 nm. In the case of PCL/drug nanofibers, as the drug concentration increased, the fiber diameter was reduced. Incorporation of 1 wt% drug resulted in the formation of fibers with average fiber diameter of 704 nm. Further addition of drug resulted in a considerable reduction in fiber diameter, and at 3 wt% drug concentration, the average fiber diameter was 521 nm.

Addition of drug resulted in the accumulation of a higher charge density on the surface of the ejected jet during electrospinning, and the overall electric charges carried by the electrospinning jet significantly increased. As the charges carried by the jet increased, higher elongation forces that could overcome the self-repulsion were brought down to the jet under the electrical field. Thus, as the charge density increased, the diameter of the fibers formed became substantially smaller and the diameter distribution of fibers became narrower.[5]

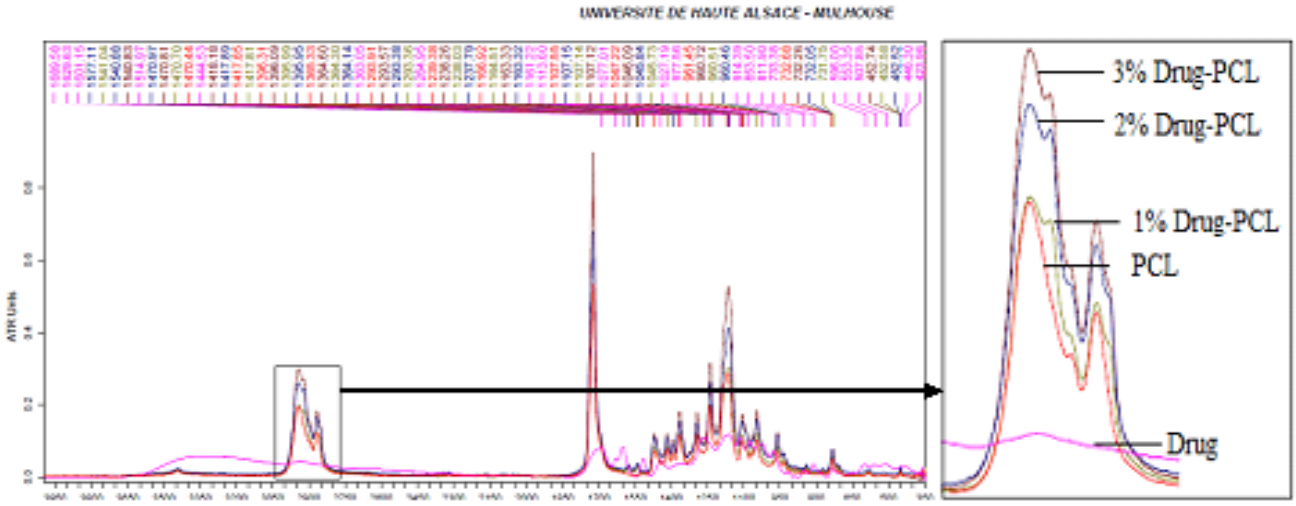
Fig.2 Fiber diameter distribution of PCL nanofiber containing various concentrations of extructed drug



3.2. FTIR analysis

ATR-FTIR spectra were conducted to study the structure of "Yemerz anchet" loaded PCL nanofiber sheets and evaluated for drug incorporation to the polymer. The scanning range was 350 to 4000 cm^{-1}. From the curve (fig.3) it is possible the see that 1723 cm^{-1} attributed to the strongest intensity of PCL which leads to adjust the intensity of the all curves at this point. So, that comparison of curves will highlight the drug contribution.

Fig.3: FTIR spectra of pure polymers, extracted drug, and drug-loaded nanofibrous scaffold



4. CONCLUSION

This study valids the extraction process of the active component issued of endemic plants from Ethiopia and highlights the abilities to include these components into nanoweb during the electrospinning process.
It can be notice that this active component does not change the general FTIR spectrum of the polymer used (PCL) but can be detected even in small quantity thank to this technic.
The next step of this study will be the "in vitro" test that will prove the anti-bacterial activity of the developed patches in order to determine the minimum of extract to be included in the nano-patche to be efficient in wound healing.

5. REFERENCE

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6. AKNOWLEDGMENT

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