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NOVEL GENTAMICIN LOADED ELECTROSPUN NANOFIBROUS SCAFFOLDS FOR WOUND HEALING: AN *IN- VITRO* STUDY

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ABSTRACT: The extracellular matrix (ECM) is the backbone in controlling cell behavior in the living organisms during the process of wound healing. The design of a suitable biomimetic nanofibrous scaffold which mimics the properties of natural ECM is a need of present time. In order to create a novel and unique wound dressing material, composite nanoscaled eudragit scaffolds loaded with gentamicin were prepared using the process of electrospinning. The nanofibers were characterized for size, size distribution, surface morphology and surface chemical structures using the Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). *In-vitro* release of gentamicin was also investigated. In vitro drug release tests confirmed that the nanofibers had pH-dependent drug release profiles. These properties indicate that the drug loaded eudragit nanofibrous scaffold has the potential to be used as a candidate in wound healing treatments.

INTRODUCTION: Wound healing and ulcer management are worldwide socioeconomic problem as they represent a large scale burden on health and resources. Scientifically, the wound may be described as a disruption of normal anatomic structure and function [1].

Wound care promotes faster wound healing and provides better functional and cosmetic implications [2]. Burns, venous ulcer, diabetic ulcer and acute injury damage the skin upto several degrees [3].

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In case of acute wounds, wound healing is orderly and timed and ultimately restores anatomic and functional integrity. On the other hand, chronic wounds have a slow and interrupted healing process and a sustained anatomic and functional result is not established [4]. The wound should be free from infection and all factors inhibiting natural healing process should be absent. The treatment becomes difficult as the bacterium is localized intracellularly since all the antibiotics are effective against the microbes *in vitro* [5].

The extracellular matrix (ECM) is the backbone in controlling cell behavior in the living organisms. The same role is played by the scaffolds in tissue engineering. Since the nanofibrous scaffolds resemble the natural ECM, they provide a suitable environment for cell attachment and proliferation [6].

These scaffolds act as a structural support for cells, aiding in the formation of new tissue. The new tissue acts as a temporary extracellular matrix and promotes the natural processes of tissue regeneration and development [7].

Recently, electrospinning has received considerable attention as an efficient and simple technique in the fabrication of nanofibrous structures [8]. Electrospun nanofibrous scaffolds offer advantages such as high surface area, high porosity and controllable pore size and so they are of widespread use in tissue engineering scaffolds, wound dressings and drug carriers.

One of the most important requirements of modern drug therapy is the controlled delivery of a drug to the site of action in the body in an optimal concentration-versus- time profile [9]. Electrospun nanofibers could increase the drug efficiency and drug solubility in aqueous solution due to their high surface-to-volume ratio [10].

This study was aimed at the investigation of the properties of gentamicin loaded electrospun nanofibrous scaffolds by scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). The scaffolds were also examined for drug release and antibacterial activity *in vitro.*

MATERIALS AND METHODS:

Materials: Eudragit, Gentamicin were purchased from FDC India Ltd., Mumbai, Polyethylene glycol (PEG-400) and methanol were procured from Merck Specialities Pvt. Ltd. Mumbai, N, N-dimethylacetamide (DMAc) was purchased from Loba Chemie Pvt. Ltd. Mumbai. All other chemicals and reagents were of analytical grade.

Preparation of gentamicin loaded nanofibrous scaffold: The gentamicin loaded nanofibrous scaffold (GNF) were prepared with eudragit polymers by electrospinning process. Typically, eudragit polymer solution with a concentration of 5% was prepared by dissolving eudragit in 5:1 (v/v) mixture of methanol: DMAc. 0.2 ml of PEG-400 was added to the prepared solution. Subsequently, gentamicin was added into the polymer solution with concentrations of 5 mg/mL and the solution was stirred at room temperature to form a homogenous solution.

The spinning solutions were loaded in 10 ml syringes and electrospun at a flow rate of 1.0 ml/h. A high voltage supply 25 kV was connected to the metallic needle (0.9 mm inner hole diameter). The ultrafine fibers were collected by a flat collector wrapped with a piece of aluminum foil with a horizontal distance of 10 cm from the needle tip. The electrospun fibers were dried at room temperature under vacuum.

Characterization of Electrospun Nanofibers:

1. **Scanning Electron Microscopy:** The surface morphologies of the prepared GNF was examined by a Hitachi-4800 scanning electron microscope (SEM). The samples were adhered to SEM studs, and then coated with gold using a sputter-coater (EMITECH K550X) for 4 min at 30 mA (**Fig. 1**).

2. **Thermogravimetric Analysis (TGA):** Thermo-gravimetric Analysis (TGA) was used to measures the amount and rate of change in the weight of a material as a function of temperature in a controlled atmosphere. TGA thermograms of Gentamicin sulphate, Eudragit RS/RL- 100 and Gentamicin sulphate loaded nanofibrous scaffold (GNF) (**Fig. 2**) were obtained using an automatic thermal analyzer system (Diamond TG/DTA 8.0, Perkin-Elmer, USA). Samples were crimped in standard aluminum pans and heated from 40 to 500^{o}C at a heating rate of 10^{0}C/min under constant purging of dry nitrogen at 20 ml/min. An empty pan, sealed in the same way as the sample, was used as a reference.

***In-vitro* Drug Release studies:** The membrane diffusion technique was used for the *in-vitro* release studies of gentamicin. The studies were conducted within a cell that was maintained at 37^{o}C under mixing conditions. The drug loaded eudragit nanofibrous scaffolds were suspended in 5 ml of aqueous buffer solution with pH 3, 7.4 and 9, respectively. The freshly prepared samples were placed onto the membrane (molecular weight cut-off of 12 000–14 000 Da, rinsed in acetone and soaked for 24 h in the diffusion medium) of the diffusion chamber, which were dialyzed in 100 ml of the diffusion media (freshly prepared saline phosphate buffer of pH 7.4, equilibrated at 37^{o}C). Aliquots of 1.0 ml of the diffusion medium were withdrawn at predetermined time from the sampling port and were replaced with an equal quantity of a fresh diffusion

medium to maintain a constant volume. To analyze the drug concentration, aliquots volume was made up to 10 ml (after treatment with ninhydrin reagent) with diffusion medium and absorbance was recorded at 566 nm against the mixture of diffusion medium and ninhydrin reagent as blank. Samples were quantitatively analyzed spectrophotometrically using Systronics 10 UV- Vis spectrophotometer at 566.0 nm. All measurements were performed in triplicate and the SD was calculated (**Fig. 3**).

Antibacterial activity of gentamicin nanofibrous scaffolds: For the zone of inhibition screening test for antibacterial activity, *Eshcheria coli* was propagated in Luria-Bertani (LB) broth for 24 h in a CO2 incubator. Bacterial suspensions were diluted and were spread onto LB agar plate. After that, the nanofibrous scaffolds were cut into pieces of diameter 1 cm and were each placed onto a lawn of *Eshcheria coli* on the agar plates and incubated overnight at 37°C. The zone of inhibition formed around the nanofibrous scaffolds was observed.

RESULTS AND DISCUSSIONS:

Surface morphology of gentamicin-loaded eudragit nanofibrous scaffolds: SEM image of GNF is shown in **Fig. 1**. The alignment of the GNF was found to be random with no bead formation, thus, making it a suitable material for wound dressings. The fibers possessed a smooth surface with the calculated average diameter of the GNF to be 355 nm. It was also found that there was no breakage of the fibers.

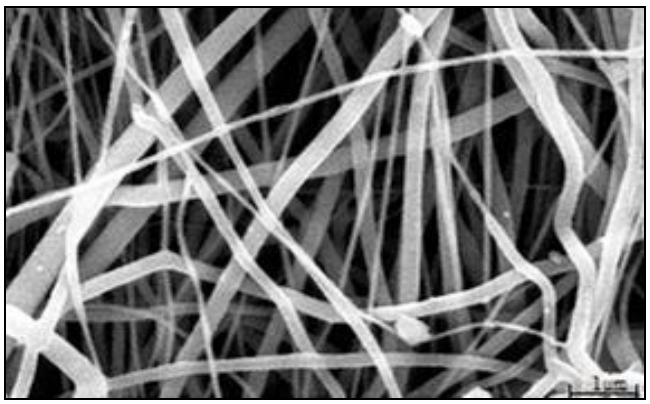

FIG. 1: SEM IMAGE OF GENTAMICIN-LOADED NANOFIBROUS SCAFFOLDS

Thermogravimetric analysis: Thermogravimetric analysis (TGA) of Gentamicin sulphate, Eudragit RS/RL- 100 and Gentamicin sulphate loaded nanofibers scaffolds (GNF) are shown in **Fig. 2**.

The results from the TGA showed two significant weight losses for the Gentamicin sulphate below 120^{o}C and at 225- 325^{o}C. While the TGA curve of Eudragit polymers showed single significant weight loss between 315- 350^{o}C. In contrast, the Gentamicin sulphate loaded nanofibers scaffolds (GNF) showed better thermal stability in comparison to Gentamicin sulphate alone. Less than 5% weight loss is observed below 225^{o}C. Furthermore, the gradual weight loss occurs up to 320^{o}C.

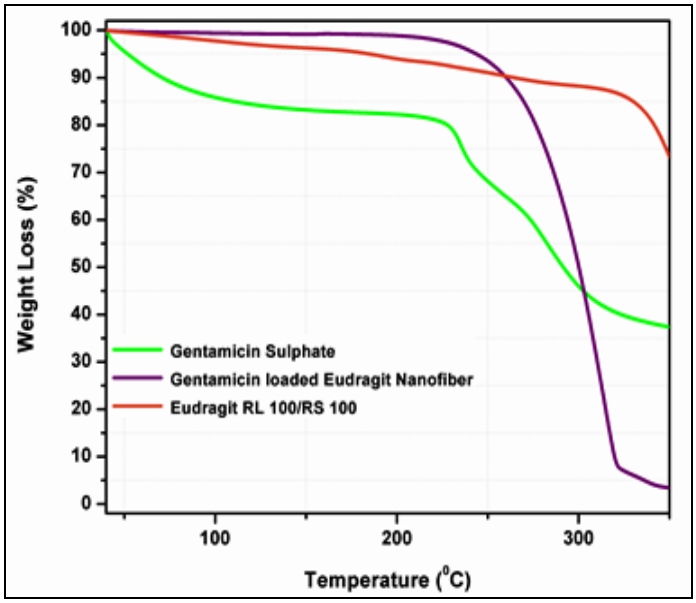

FIGURE 2: THERMOGRAVIMETRIC ANALYSIS

Drug release kinetics from Eudragit scaffolds: The gentamicin release curves from GNF at different pH are illustrated in **Fig. 3**. *In vitro* drug release studies revealed constant drug release and no burst effect was observed indicating that the drug was homogeneously dispersed in the eudragit polymeric matrix and there were no significant amount of drug adsorbed onto the surface of nanofibers.

At pH 7.4, gentamicin was released slowly from the nanofibrous scaffold and only 37.2% of the total bound gentamicin sulfate was released in 12 hours. However, 91.75% and 72.8% of the drug was released in acidic and basic conditions respectively after 12 hours. This release is much higher than that released at pH 7.4. This is so because the hydrogen bonding interaction between gentamicin and the eudragit nanofibrous scaffold is strongest at the neutral pH. Whereas at pH 3 and 9, there is a comparatively weaker hydrogen bonding interaction, so the higher amount of gentamicin was released at pH 3 and 9.

There was higher percentage release of gentamicin at pH 3 compared to pH 9 because the hydrogen bonding interaction formed under basic conditions was stronger than that under acidic conditions.

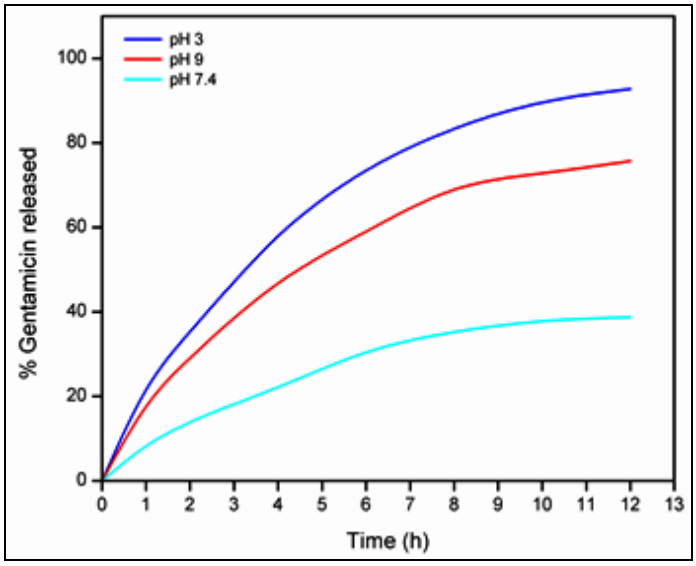

FIG. 3: THE RELEASE PROFILE OF GENTAMICIN SULFATE ON EUDRAGIT NANOFIBROUS SCAFFOLD AT DIFFERENT pH VALUES.

Antibacterial activity of Gentamicin-loaded Eudragit Nanofibrous Scaffolds: Antibacterial activity of gentamicin-loaded eudragit nanofibrous scaffolds was investigated against *E. coli* bacteria. Good zone of inhibition was found around the nanofibrous scaffolds which showed that the growth of bacteria was inhibited around the scaffolds. This inhibition of bacterial activity could be attributed to the release of gentamicin from the scaffolds which inhibited the growth of bacteria in that region. Thus, the gentamicin released from the scaffolds showed a good inhibitory effect on the growth of bacteria and killed the microorganism.

CONCLUSIONS: In this study, we successfully prepared eudragit nanofibrous scaffold loaded with gentamicin. Surface morphology of the nanofibrous scaffolds was smooth with no breakage of the fibers. It was verified from the in vitro dissolution studies that the release profile of gentamicin was dependent on the pH of the buffer solutions.

The percentage release of gentamicin was higher at acidic pH as compared at basic pH. The fibers were also found to possess a strong antibacterial activity, thus, showing a great potential to be used as a wound healing material.

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