Electrospun gelatin sheet reinforced with anti-hypertensive drug for wound healing application

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Abstract

Testing the potential of an electrospun fibrous sheet made with gelatin loaded with the ACE angiotensin converting enzyme inhibitor perindopril (PE-G) was the aim of the investigation. Physico-chemical property analysis was done utilizing Fourier transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX). Cell migration properties of PE-G in comparison to gelatin scaffold are employing dermal fibroblast cells in vitro. The porosity pattern of the non-woven gelatin sheet was not altered in 1% PE-G, but it was by 3% and 5% PE-loaded gelatin without changes in the elements.

FTIR measurements verified the existence of PE and gelatin functional groups in the PE-G scaffold. According to XRD examination, the PE-G fibrous sheet's percentage of crystalline and amorphous nature was altered. Compared to PE-loaded fibrous sheets, gelatin showed a significant cell migratory population. PE-loaded gelatin may be a good option for wound healing applications.

Keywords: Angiotensin-converting enzyme inhibitor, Perindopril, Tissue engineering, Wound healing.

Introduction

Wounds are caused by loss of integrity of skin due to many factors such as trauma, burns, surgery, thermal injuries, chemical burns and lacerations leading to socio-economic issues in all societies. Superficial wounds heal spontaneously as the human skin has the ability to regenerate but in the case of severe injuries, this healing potential is not enough and there is an immense need for the protection of the wound area until the skin regeneration process is completed[21].

The majority of wound management methods used currently such as irrigation, debridement, antibiotics, proteolytic enzymes and tissue grafts, have limitations, the most important of which are their invasiveness, high cost and long-term treatment for chronic wounds[15]. Wound healing is a dynamic, spontaneous process that involves the interplay of cytokines, blood, extracellular matrix and growth factors. The main goals of wound care are to stop the bleeding and to keep the patient away from getting an infection[6]. Therefore, there is a need for an optimum wound dressing to accelerate the wound healing process[10]. A wound dressing with suitable characteristics including biocompatibility and hydrophilicity offers one of the best ways to treat these types of wounds.

Gelatin stands out as a prominent biopolymer commonly employed in crafting wound dressings while also finding significant utility in biomedical and pharmaceutical domains. It is one of the most important biopolymers for the creation of electrospun nanofiber mats for wound healing applications. It plays a crucial role in the field of tissue engineering due to its natural similarity to the extracellular matrix (ECM) found in human tissues and organs[13]. Gelatin is optimally suited for wound dressing, providing a fluid absorbent physical barrier and moist scaffold for skin. Additionally, due to their ultra-low endotoxin levels, mechanical strength and microstructure, gelatin provides the ideal carrier for active molecules used in bioactive dressings to accelerate wound healing without risking a negative immune reaction[19].

The drawbacks of gelatin include lack of antibacterial properties, weak mechanical properties and quick degradation in *in vivo* application. Prodrugs that function as angiotensin-converting enzyme (ACE) inhibitors, such as perindopril erbumine (PE), are commonly used to treat cardiovascular disorders, especially systemic hypertension. It has also been reported that ACE inhibitors play an important role in tissue fibrogenesis, a wound healing and repair process. PE, as arginine salt, exhibits significant shelf life and stability under high temperatures and relative humidity[7].

Further it has been documented that angiotensin II encourages blood vessel formation and cell proliferation. Based on these data, one may hypothesize that ACE inhibitors at modest doses might promote wound healing more successfully. Research has demonstrated that a VEGF-dependent route enables a very low dose of perindopril to cause an early and long-lasting effect on revascularization in ischemic tissue[17].

Material and Methods

Electrospinning: A blend of glacial acetic acid (Emplura®, Merck, Mumbai, India) and DMSO (Sigma Aldrich, Mumbai, India) in the ratio of 93:7 was utilized as the solvent to dissolve the polymer. 19% gelatin (Himedia, India) and 1%, 3% and 5% of PE (Alsachim, France) (PE-

G) were dissolved in the solvent system along with the control, 19% gelatin without PE. A syringe was filled with the polymer solution and placed on a syringe pump (Flogard+, Trivitron Healthcare, India). A stainless-steel needle was fitted into the syringe and connected to a high-voltage power supply unit (Kwik Lite Premier Combines) and operated at 20 kV.

Electrospinning was performed at humidity of 50–55% and a temperature of 22–25 °C. The flow rate was set at 2.5ml/hr and by placing a collector plate 20 cm from the needle tip, the fibers were collected by an aluminium foil on the collector surface. For crosslinking, the scaffold was treated with 50mM 1-(3-dimethylaminopropyl)- 3-ethylcarbodiimide (EDC) (SRL, Mumbai, India) for 12-14 hours and then in 0.1M sodium dihydrogen phosphate (SRL, Mumbai, India) for 2 hours. The crosslinked scaffolds were stored with antibiotics before use.

Sterilization: After the crosslinking process, the scaffolds were retrieved and subjected to sterilization through 70% ethanol for 30 minutes in a rocker, finally washed with autoclaved water twice and stored at 4°C until further analysis.

Scanning Electron Microscopy-Energy Dispersive X-ray spectroscopy (SEM-EDX): SEM-EDX was performed to analyze the morphology of the fibers and elemental composition using the Scanning electron microscope (JEOL-JSM IT 800, Germany).

Fourier Transform Infrared Spectroscopy (FTIR): To determine the chemical functional groups present in the scaffolds, FTIR analysis was conducted using a Spectrum 400 instrument (Bruker Alpha 2, Germany). KBr powders and the sample were mixed in a 1:10 ratio and were pressed into a tablet form under 10 bar pressure. The spectra were recorded within the range of 400–4000 cm^{-1} with a scan rate of 4 cm^{-1} over 130 scans.

X-ray Diffraction (XRD): Crystalline properties of the fibrous sheet were analyzed and the XRD patterns were collected using a D8 Advance Powder X-ray diffractometer (Bruker AXS GmbH, Germany) and were utilized to record the Bragg peak XRD patterns. The diffractometer operated with a Ni-filtered monochromatized CuKα radiation (λ = 1.54056 Å) at 40 kV and 40 mA and the temperature was maintained at 25 °C. The scanning rate used was 0.1 degrees s^{-1}. The peak diffraction patterns were plotted in the range of 5 to 100 2θ°.

***In vitro* cell migration assay**: Fibroblast cells (LifeCell, India) were cultured in Dulbecco's modified Eagle's medium (Himedia, Mumbai, India) supplemented with 10% Fetal bovine serum (FBS) (Gibco, USA) and 1% Penicillin-Streptomycin-Amphotericin (Lonza, USA) solution at 37 °C in a humidified atmosphere of 5% CO_2. Briefly, fibroblast cells (10,000 cells/well) were seeded onto a 24-well plate. A vertical cell-free scratch was manually created in the middle of the monolayer by using a 1000 µL sterile tip and washed with PBS to remove cellular debris prior to adding culture media. The wound closure rate and the cell migration were monitored over time (0h and 48h). Images of the scratches were captured at 48h post-scratching using a fluorescent microscope (Nikon, Eclipse TE2000-S).

Results

In fig. 1a, gelatin control as a fibrous sheet is depicted in SEM images and PE-G (1%, 3% and 5%) is depicted in figs. 1b, 1c and 1d. The rough surface of the scaffolds is also visible in images. The surface of both gelatin and 1% PE-G scaffolds showed an interconnected ribbon, like network with an open pore structure with an average macropore size of about 1 µm. In comparison, with the increase in the percentage of PE from 3% and 5%, the pattern of pore size decreased. All nanofibers displayed a consistent distribution and were randomly orientated with no bead formation. The elemental analysis (Fig. 2a-d) of 1% PE-G scaffolds revealed peaks of carbon and oxygen, nitrogen and sulfate and the peaks in the 3% and 5% PE incorporated fibers showed not much difference in the levels of elements. The blending of PE with gelatin resulted in the shifting of peaks and functional groups of PE were also masked by gelatin bands.

The peak at 1079 cm^{-1} corresponds to the C-N vibration band and N-H was observed at 3293 cm^{-1}. The band at 1340 cm^{-1} was due to the presence of carboxylate stretch. The bands associated with gelatin, such as amide-I, II and III, were observed at 1630 cm^{-1}, 1533 cm^{-1} and 1235 cm^{-1}. Peaks spanning the range of 1449 cm^{-1} to 1330 cm^{-1} are attributed to the symmetric and asymmetric bending vibrations of methyl groups (Fig. 3)[2]. Strong diffraction peaks between 10-25 2θ indicated a high degree of PE crystallinity. Gelatin, used in the electrospinning, indicated peak reflections from 40-45 2θ, 60-70 2θ and 75-80 2θ. The percentage of crystalline nature of PE powder was 64.8% and amorphous was 35.2%, gelatin was 47.6% crystalline and 52.4% amorphous. In comparison, the PE-G fibrous sheet was crystalline- 46.3% and amorphous- 53.7% (Fig. 4).

Like the surface area of the scaffold, the crystalline and amorphous properties have a role in the mechanical properties of the scaffold. Cell migration was noted in the gelatin-treated scratched region as indicated in fig. 5a. Likewise, PE-G scaffolds also showed cell migration and the region of migration was pointed out across the scratched area in 3% and 5%. In 1% PE-loaded gelatin, though migration was noted, some dead cells were observed, as indicated by the arrow (Figures 5a-d).

Discussion

Natural materials are the preferred choice for making fibrous scaffolds. Hence, gelatin was chosen in the study because of its biocompatibility and ability to mimic the components of natural skin tissue[4,20]. Previous studies have shown diverse

polysaccharides like collagen and sodium alginate to be explored for fabricating fibrous scaffolds[11,18]. Natural scaffolds made of gelatin are less likely to induce an immune response or inflammatory response.

Among the several techniques for creating nanofibrous membranes, electrospinning exhibits great promise since it enables the inclusion of medications or drugs, which can then be released at a regulated pace across various time intervals[9]. Electrospinning is a straightforward but efficient method to produce fibrous materials in the micro to nano-scale range that can be applied to a variety of tasks including wound dressing, medication administration and tissue regeneration[8].

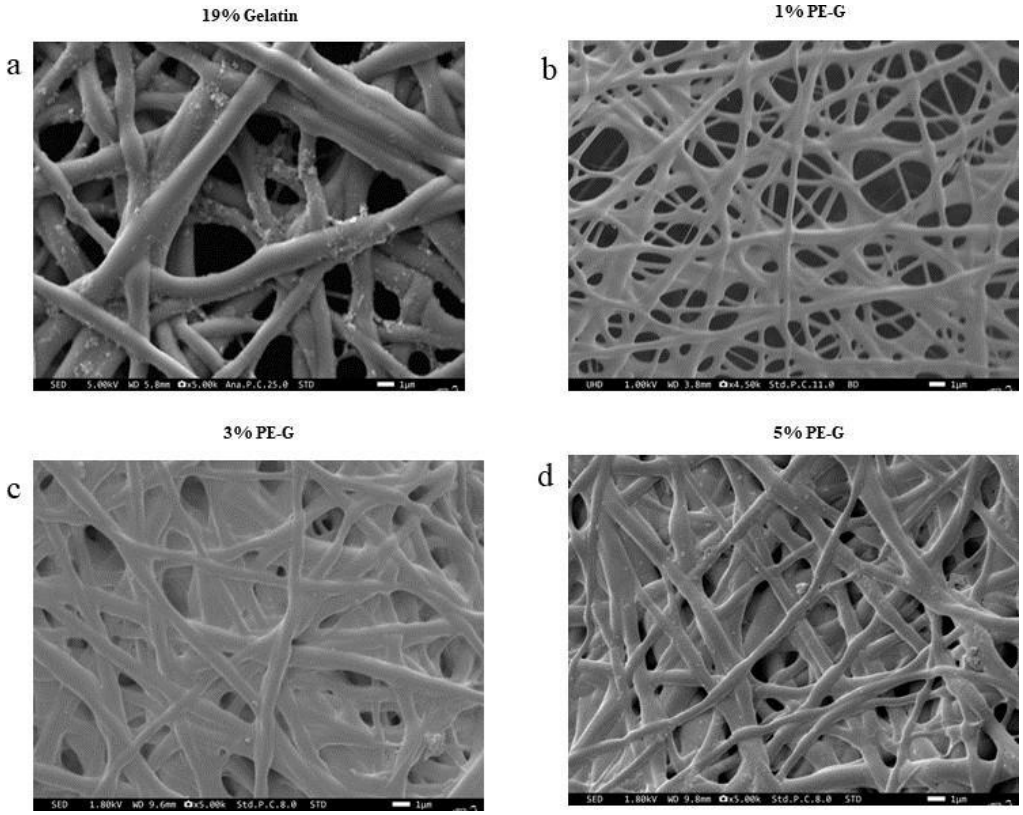

Fig. 1: SEM images of 19% gelatin and different percentages of PE-G; (a) 19% gelatin, (b) 1% PE-G, (c) 3% PE-G and (d) 5% PE-G

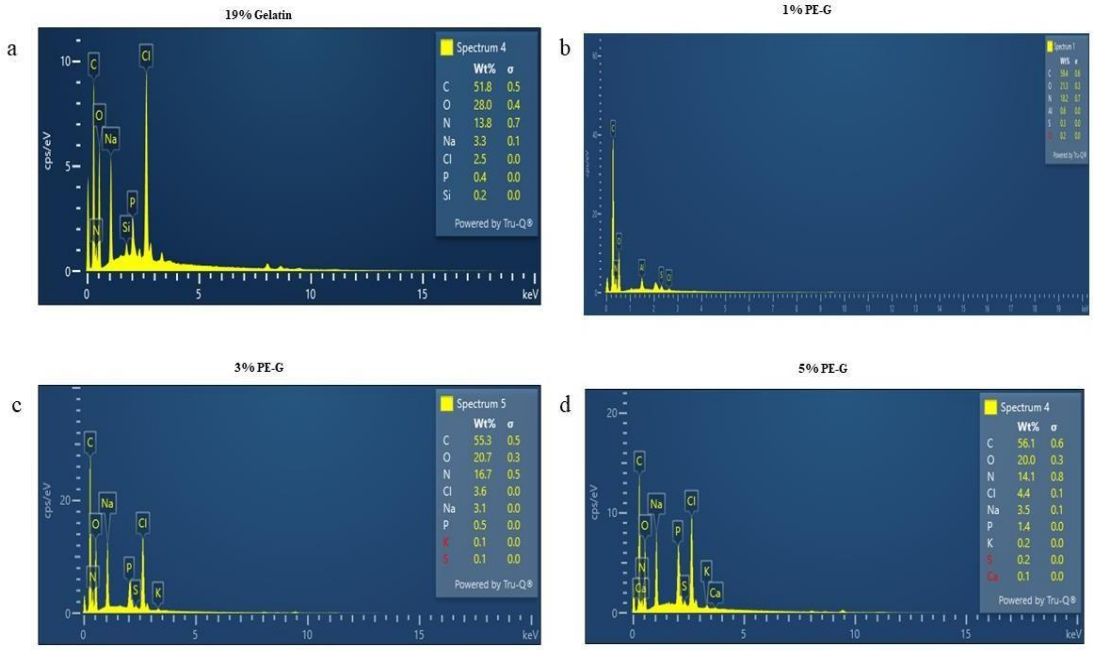

Fig. 2: EDX images of 19% gelatin and different percentages of PE-G; (a) 19% gelatin, (b) 1% PE-G, (c) 3% PE-G and (d) 5% PE-G

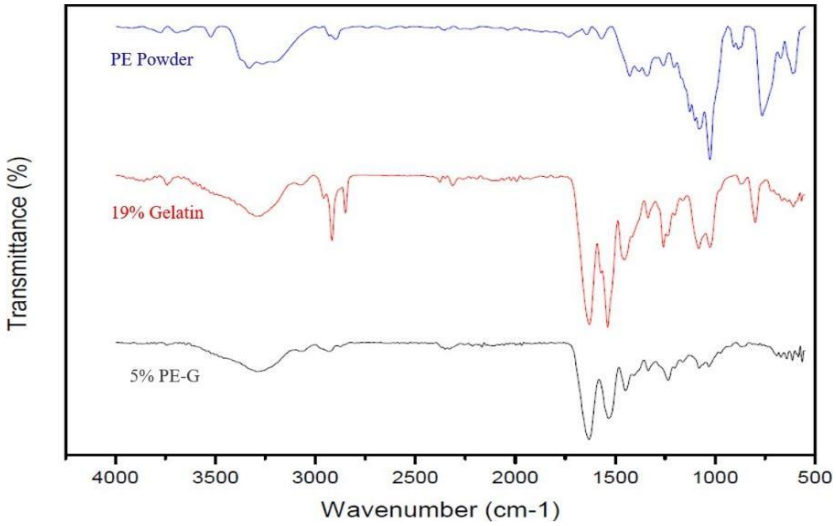

Fig. 3: FTIR spectra of PE powder (blue), 19% gelatin (red) and 5% PE-G (black)

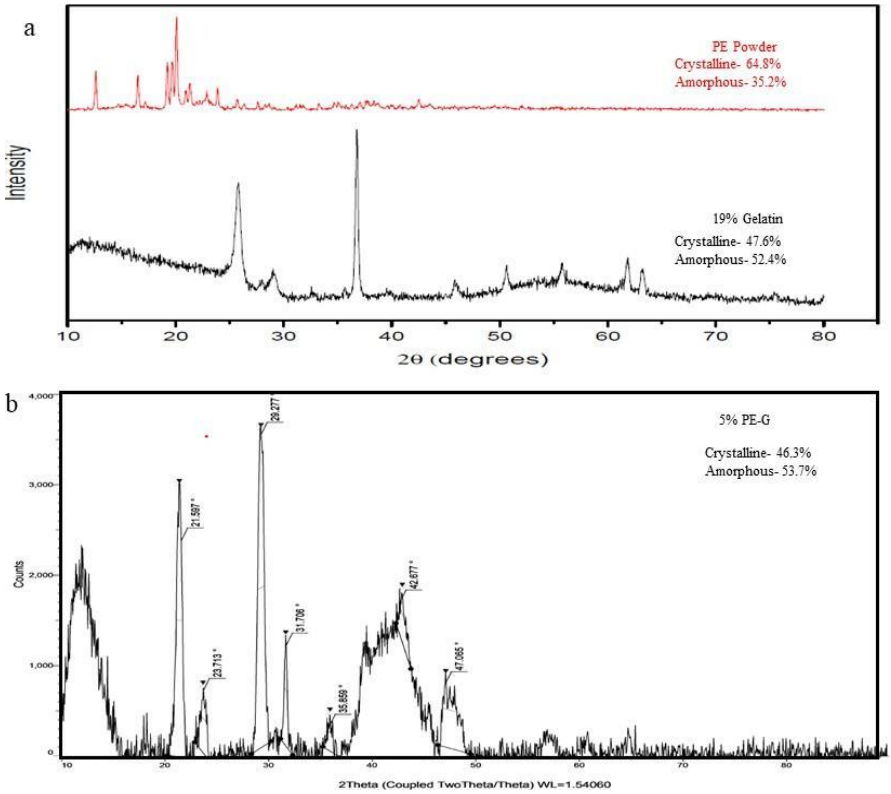

Fig. 4: XRD patterns; a-PE powder (red) and 19% gelatin (black) and b-5% PE-G

Studies have shown the surface of the scaffold is critical in cell adhesion properties and it was evident that this fibrous scaffold surface was suitable for cell attachment and provided the required physiological environment[1,22]. While the percentage of crystallinity of PE powder was found to be 64.8% and 35.2% of amorphous nature, gelatin showed 47.6% crystallinity and 52.4% of amorphous nature. PE-G fibrous sheet was observed to be 46.3% crystalline in nature and 53.7% amorphous in nature, indicating that the porous surface of gelatin scaffolds was altered by PE addition. Like the surface area of the scaffold, the crystalline and amorphous properties have a role in the mechanical properties of the scaffold[3,16]. Studies have shown that micro- and nano-hierarchical structures influence the cell's behaviors and the wound healing capacity. Thus, we have attempted to check whether PE would improve cell migration of dermal fibroblast cells *in vitro*[5]. Results demonstrated the presence of dead cells that were, however, insignificant, while the reason for their presence seems to be elusive and needs further evaluation. Earlier reports have indicated that ACE inhibition is thought to be one of the mechanisms in reducing fibrosis of tissue by decreasing the production of angiotensin levels. This idea has led the researchers to identify various ACE inhibitors that could be advantageous for the wound healing pattern in skin wounds. Pre-clinical studies have also shown that inhibition of ACE would reduce hypertrophic scars in the tissue and promote wound healing[12,14].

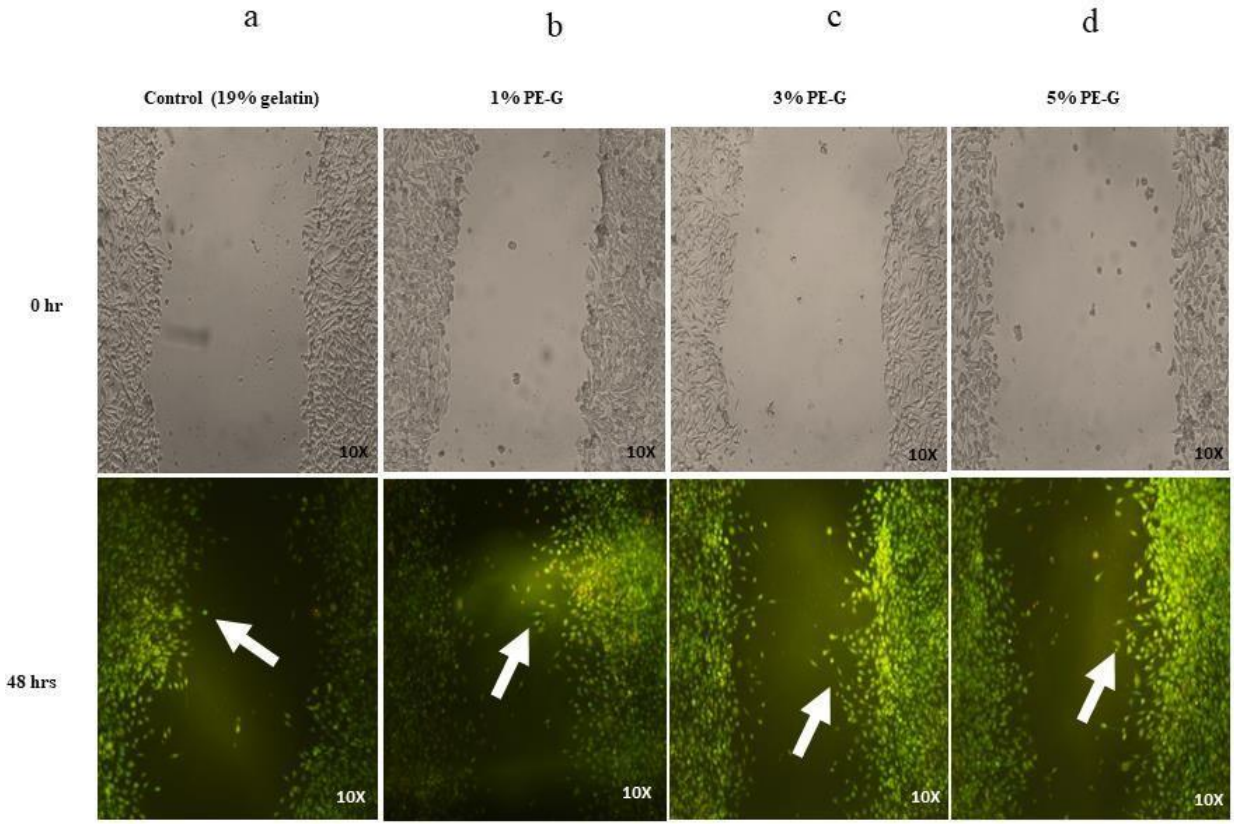

Fig. 5: ***In vitro*** **cell migration assay demonstrating that migration into the cell free region in the presence of 1%, 3% and 5% PE when compared to control (19% gelatin); Cell migration on (a) control (19% gelatin), (b) 1% PE-G, (c) 3% PE-G and (d) 5% PE-G at time points 0hr and 48hr**

Conclusion

This pilot model study showed that loading PE and gelatin together effectively produced a non-woven fibrous sheet. The amorphous and crystalline nature of the material changed consistently with the topography of the scaffold, according to the results. The experiment used for migration demonstrated cell movement without appreciable cell death. The absence of an animal-related healing model was the study's main disadvantage. Overall, based on the results, this scaffold may be investigated further and targeted for use in biomedical applications, particularly in the area of wound healing.

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