Thermoplastic Polyurethane/Plavix Electrospun Saffolds for Vascular Grafts Application

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ABSTRACT
Synthetic vascular grafts with a tiny diameter are particularly vulnerable to thrombosis when utilized for bypass treatments. One promising option for addressing this unmet clinical need is the research and development of bioresorbable vascular scaffolds that can release antithrombotic drugs locally and over a prolonged period within the vascular lumen. To achieve this goal, we present the development and characterization of aPlavix -loaded electrospun Thermoplastic Polyurethane (TPU) scaffold for vascular grafts. Three aspirin dosages were investigated (0.2, 0.6, 1) % from Plavix value. All of the platforms had a fibrous structure. Measurement of theViscosity-Shear Rate Relationship,Surface Tension, Electrical Conductivity, contact angle, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (FESEM), atomic force microscopy (AFM), were used to complete the characterization. In addition, Blood compatibility of the final nanofibers was evaluated using the activated partial thromboplastin time APTT and partial thromboplastin time PT,As a result of the viscosity of the samples decreased with increasing Plavix concentration and also with an increasing shear ratefor TPU/Plavix, the surface tension increased with increment of the cohesiveness. For the contact angle test, the produced nanofiber is hydrophilic. TPU and Plavix form a hydrogen bond, and the FTIR demonstrates this interaction. In contrast to the pure TPU control, the FESEM examination of the manufactured scaffolds revealed a random morphology of nanofibers. Using AFM analysis, we showed that the surface smoothness of nanofibers was elevated in comparison to the control. Results from activated partial thromboplastin time (APTT) and partial thromboplastin time (PT) assays showed that the manufactured scaffolds were anticoagulant by delaying the activation of a clot. In this study, researchers hypothesized that recently created nanofibers would precisely match desirable vascular graft application features.

Keywords: Electrospun, TPU, Plavix, thrombosis prevention, Nanofibers

1. INTRODUCTION

Vascular grafts are used for bypass procedures of coronary and peripheral arteries when percutaneous transluminal angioplasty or stenting procedures cannot assure long-term vessel patency. For this aim, autologous arterial and venous grafts remain the best clinical option, though are known to be affected by several limitations mainly related to pre-existing vascular disease, prior surgery, limited length, or poor quality. Commercially available biostable synthetic vascular grafts made of thermoplastic polyurethane (TPU) have failed for small-diameter (<6 mm) vessel replacement due to an early graft occlusion, since thrombosis, together with intimal hyperplasia, remains one of the major causes of poor patency. To prevent early graft occlusion, patients are treated with systemic antithrombotic drugs such as anticoagulants and platelet inhibitors[1].

This study made adjustments to a commercially available preprocessed polyurethane [polyether-based thermoplastic polyurethane (TPU)]. They can be put to use in the biomedical field, and their mechanical qualities, chemical stability, and ease of processing are all top-notch (e.g., as catheters and artificial veins). High mechanical flexibility and other qualities (no plasticizers, good heat resistance) led to this material's selection. With its high biocompatibility, thermoplastic polyurethane (TPU) has found several medical applications, including a ventricular frame and heart valve. Thermoplastic polyurethane (TPU) polymers are in a class by themselves since they offer so many attractive characteristics. The tetraetilamoniumbromide, N, N-dimethylformamide, dimethylacetamide, ethanol, tetrahydrofuran, and their respective combinations are all suitable solvents for dissolving TPU. Heating TPU to around 120 °C produces a viscous condition[2].

Plavix is a common anti-inflammatory drug and is widely prescribed as an anti-platelet to prevent cardiovascular events. Although Plavix's main administration route is oral, this route has poor bioavailability (40–50%) and its prolonged use is associated with gastrointestinal mucosa ulcers and gastrointestinal hemorrhaging in severe cases. In the literature, there is evidence that Plavix parenteral administration (non-oral) can reduce gastrointestinal side effects. An alternative to oral administration is developing Plavix local delivery systems by loading the Plavix into polymeric implants for cardiovascular applications, such as stents, scaffolds, and gels[3].
Several techniques can be considered for the production of vascular graft systems, among them electrospinning represents an easy and cost-effective method to produce nano- or micro-fibrous polymeric scaffolds of desired shape and dimension. Fiber diameter, architecture, void size and interconnectivity, microstructural and mechanical properties can be properly tailored to mimic the natural extra-cellular matrix (ECM) of blood vessels and to affect the drug from the selected material[4].
In this regard, loading polymeric vascular grafts with Plavix, an efficacious, relatively safe, widely available, inexpensive, and easy-to-use antiplatelet agent, could represent a novel, easy and time-saving way to improve hemocompatibility of polymeric vascular substitutes. Therefore, the purpose of this study was to fabricate small diameter Plavix -eluting electrospun TPU vessels as a potential model for vascular grafts aimed to inhibit thrombus formation and to evaluate their mechanical properties, in vitro drug release mechanism and platelet anti-adhesion properties as a function of the drug content. To the best of our knowledge this is the first report regarding the feasibility of electrospinning Plavix -loaded TPU scaffolds resembling the natural ECM of blood vessels.

2. Experimental part

2.1 Materials

TPU (Mn = 30,000 g/mol) and N, N-DMF were supplied by Sigma.Plavix was supplied by Sigma-Aldrich. Phosphate buffer solution (PBS, pH 7.4) tablets were purchased from Invitrogen Corporation. All materials and reagents were used as received.

2.2 Preparation of electrospinning solutions

Three polymeric solutions were prepared. TPU solution was prepared by dissolving 2g TPU in 8 ml DMF and mixing at RT for 60 min. Plavix was prepared by dissolving(0.2,0.6,1)%for three present of Plavixrespectively in TPU solution with continuous mixing at RT for 30 min to prepare the polymeric solution which is then pumped by electrospinning technique to create ananofiber bead.

2.3 Electrospinning process

After preparing solutions, an electrospinning process occurred to the solution to get the nanofiber textiles. The process conditions used were the applied voltage was 19 kV, the tip-collector distance was 20 cm, the temperature was (25-37)℃, the rotation speed was 600 rpm, and the flow rate was 1ml/hr.

3. Tests

The viscosity test was done using "Brookfield DV-III Ultra Rheometer. The surface tension of the solutions was measured with anSL 200C - Optical Dynamic I Static Interfacial Tensiometer. Electrical conductivity test by using an electrical conductivity device (model HANNA instruments - EC 214 conductivity Meter.FTIR test was carried out using (IRAFFINITY-1) (Shimatzu) to check the structure of nanofiber. Contact angle of the nano fiber was measured (SL 200C - Optical Dynamic I Static Interfacial Tensiometer). The morphology of the nanofibers was evaluated using field-emission scanning electron microscopy (FE-SEM) (MIRA3 TESCAN- FRANCE). Atomic Force Microscopy (AFM) is a surface measurement technique based on a tip's interaction with the surface of the sample.The release of Plavix tested for solutions samples byusing UV-visible(CECIL 2700 computerized spectrophotometer). The antibacterial was measured for solutions and data were statically analyzed using the GraphPad prism program. APPT and PT the time taken for the formation of the white fibrous clot was noted using a chronometer.

4. Results and Dissections

4.1 Solutions Results

4.1.1 Viscosity-Shear Rate Relationship

This relationship between viscosity and shear rate refers to what is called apparent, or shear viscosity. In Newtonian fluids, this value doesn't change, but with non-Newtonian fluids, apparent viscosity is directly affected by the shear rate.

The viscosity of a liquid is related to the ease with which the molecules can move concerning one another. Thus, the viscosity of a liquid depends on the: strength of attractive forces between molecules, which depend on their composition, size, and shape.It is greater with larger than with smaller molecules, with elongated than with spherical molecules.
The shear rate is defined as the gradient in velocity, that is, the difference in velocity between the two surfaces containing the fluid, divided by the distance between them.
According to Figure1A, it is clear that the neat TPU solution exhibits a shear thinning phenomenon, where its viscosity decreases as the shear rate is increased. This refers to the rearrangements in the fluid microstructure in the plane of the applied shear. This is because the TPUis composed of low molecular weight polymers and due to the low concentration of this solution. However, in addition, because the TPU material itself is soft. This means that, as the shear stress increases, the fluid begins to deform more easily and shows to be less viscous.As for adding Plavixas shown in Figures (B, C, D) three percent (0.2, 0.6, 1) % from Plavixrespectively. The viscosity of the samples decreased with increasing Plavix concentration and also with increasing shear rate. It was also noted that samples containing Plavix appeared to be less brittle than TPU. Plavixis one of the most commonly used drugs in the world and is widely utilized for the prophylaxis and treatment of atherosclerotic vascular disease and arterial thrombotic disorders due to its ability to decrease the viscosity of whole blood and reduce clotting due to platelet aggregation[5].

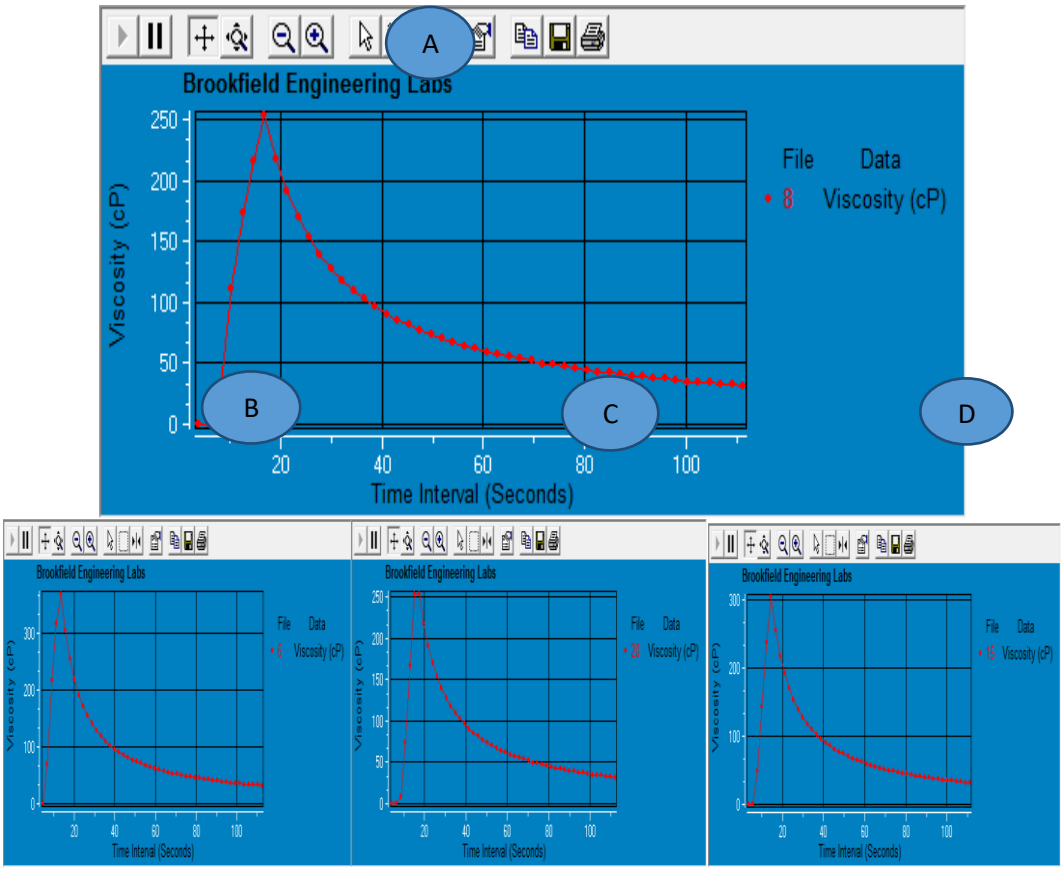

Fig 1: Relationship Between Viscosity And Shear Rate For The(A)Neat TPU And (B), (C) ,(D)For TPU/PlavixFor Three Percent (0.2, 0.6, 1) % From Plavix.

4.1.2 Surface Tension and Electrical Conductivity Results

The surface tension showed in the table 1 for neat TPU and TPU/Plavix is the property of the liquid that shows the strong cohesiveness of the liquid molecules, indicating the dissimilarity of the phases at the interface. Generally, as the content of any used additive increased, the surface tension increased due to the increment of the cohesiveness of the solution molecules, which leads finally to enlarge the dissimilarity and the phase separation among the solution components[6].
The conductivity of the solution has a strong impact on the electrospinning process and fiber morphology. Also, the increasing voltage will accelerate the electrospinning jet and this may result in a greater volume of solution drawn from the tip of the needle[7].

Table 1: Surface Tension Values and Electrical Conductivity Values of the Prepared Samples

Sample	Ratios %	Surface tension (mN/m)	Electrical conductivity (mS/cm)
Neat TPU	10%	34	2.03
TPU/Plavix	0.2%	34.65	1.95
	0.6%	34.96	1.99
	0.1%	36.10	2.08

4.2 Contact Angle Measurements

The addition of Plavix values (0.2, 0.6, 1) % to neat TPU a smaller water contact angle of (55°,48°,43°) for three present from Plavix respectively while for TPU the contact angle is 71° which confirms the successful preparation of a highly hydrophilic surface for cell interactions. The use of substrates with a hydrophilic surface is essential for proper cell spreading and morphology. These materials should ensure extensive interactions between the cell membrane and their surface[8]. The contact angle also decreased with increasing Plavix amount, and thesurface has become more hydrophilic. According to the literatures, lower surface hydrophobicity originating from high surface energy could lead to faster cell activation, spreading, and differentiation. Thus the appropriate loaded amount of Plavix could convert hydrophobic membrane surfaces into hydrophilic surfaces, which was beneficial for cellular behavior [9].

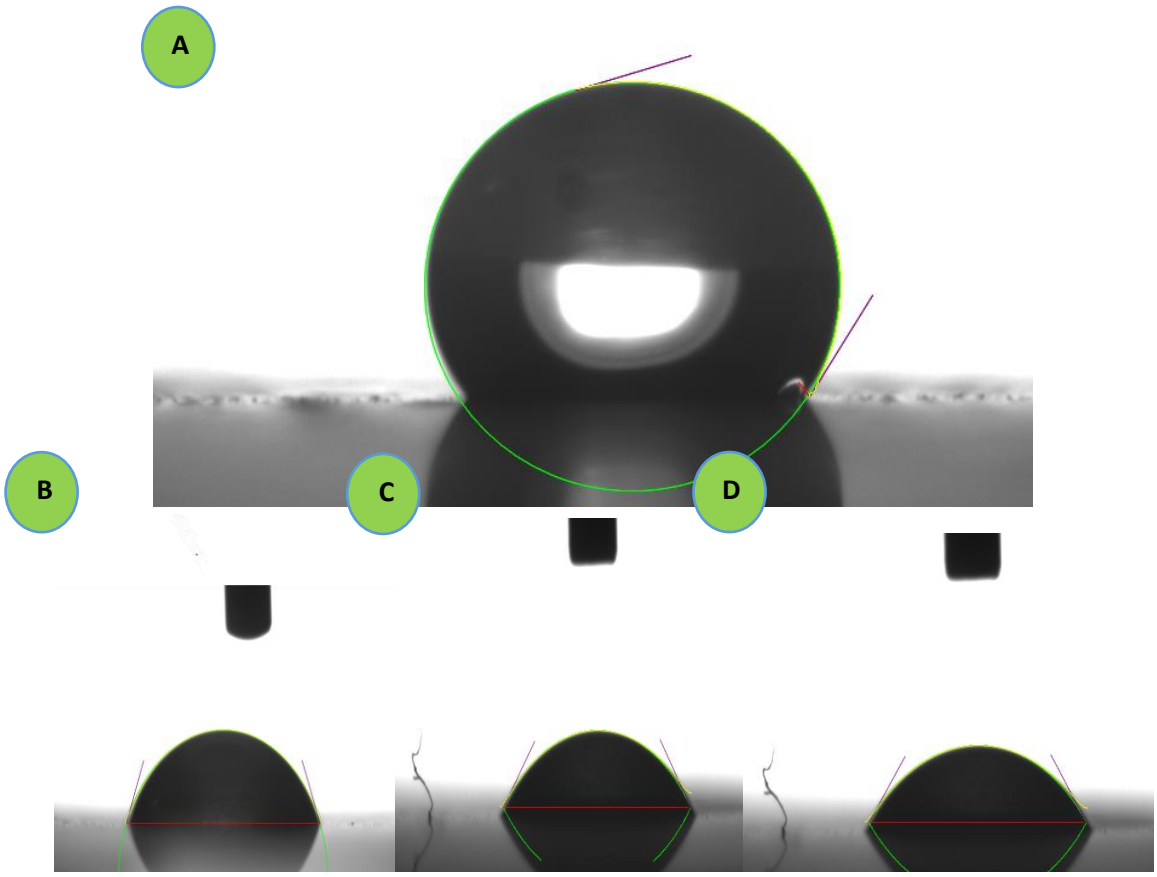

Fig 2: Contact Angle Images Of (A) Neat (TPU), (B) TPU/ (0.2) % PlavixNanofibers. (C) TPU/ (0.6) % Plavix Nanofibers. (D) TPU/ (1) % Plavix Nanofibers.

4.3 Ftir Results

Figures 3 present spectra of neat TPU and TPU/Plavix nanofiber. It is of interest to note that there are no significant differences in the fingerprinting region between them and the results were confirmed by the appearance of the same characteristic absorption peaks in the spectrum of the physical mixture without any changes in their position figures which, indicated an absence of chemical interaction[10].

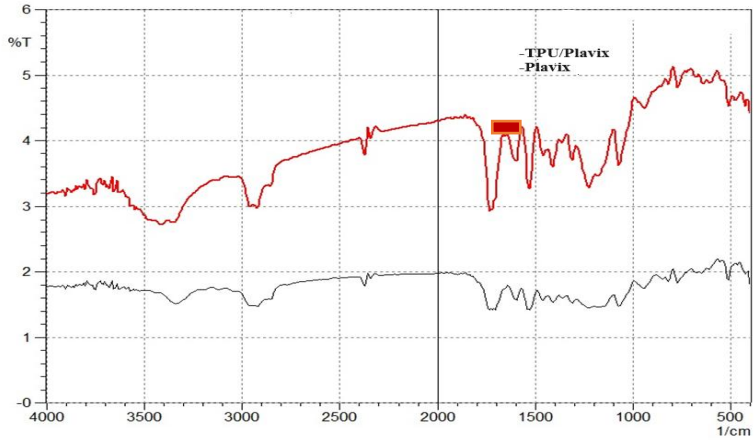

Fig 3: FTIR Spectrum of (A) neat TPU and (B) TPU/Plavix Nano fibers

Table 2: The Transmission Bands ofIR Spectrum Characteristics ofTPU/Plavix

Type of bond	Neat TPU cm^{-1}	TPU/Plavix cm^{-1}
NH-stretching	3330 cm^{-1}	3328.50 cm^{-1}
CH_2-stretching	2861 cm^{-1}	2860.93 cm^{-1}
CH_2-stretching	2934 cm^{-1}	2930.62 cm^{-1}
C=O stretching	1726 cm^{-1}	1720.59 cm^{-1}
NH-stretching	1528 cm^{-1}	1529.11 cm^{-1}

4.4 Morphology Test

Field emission scanning electron microscope(FESEM) For neat TPU and TPU/Plavix, the average diameter decreased from 870 ± 90 to 650 ± 130 nm respectively. These results were mainly attributed to Plavix powders increasing the charge density on the surface of the jet flow. Thus, the charges could suffer from drafting force in overcoming the surface tension of the solution. In this case, on the other hand, the fiber diameter obtained might be smaller[11].In several studies, it was concluded that a higher applied voltage leads to increased ejection of polymer fluid in a jet but there have been also reports of an initial decrease in fiber diameter. it can be concluded that an increase in electrical conductivity leads to a decrease in average fiber diameter[12].

Plavix is used to relieve mild to moderate pain, reduce fever, redness, and swelling, and help prevent blood from clotting because inhibits the body's production of prostaglandins. Prostaglandins are hormone-like substances that are involved in the regulation of varied processes such as pain, fever, inflammations, and thrombosis. Prostaglandins elicit signals of pain and so by obstructing their synthesis, no pain can be felt[10].

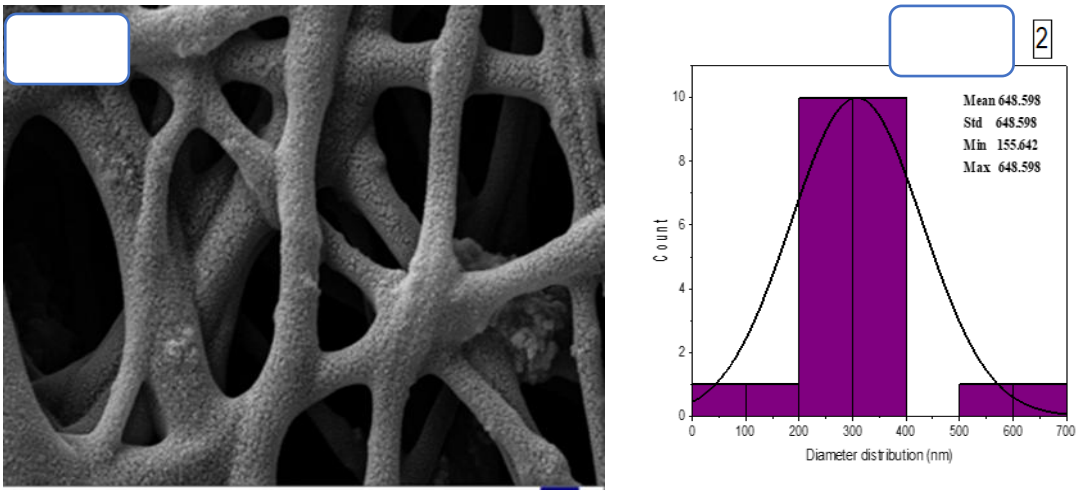

Figure 4 (A): FESEM Image of Neat TPU with 3500 Magnification Powers (B) Diameter Distribution Of Neat TPU Nanofiber

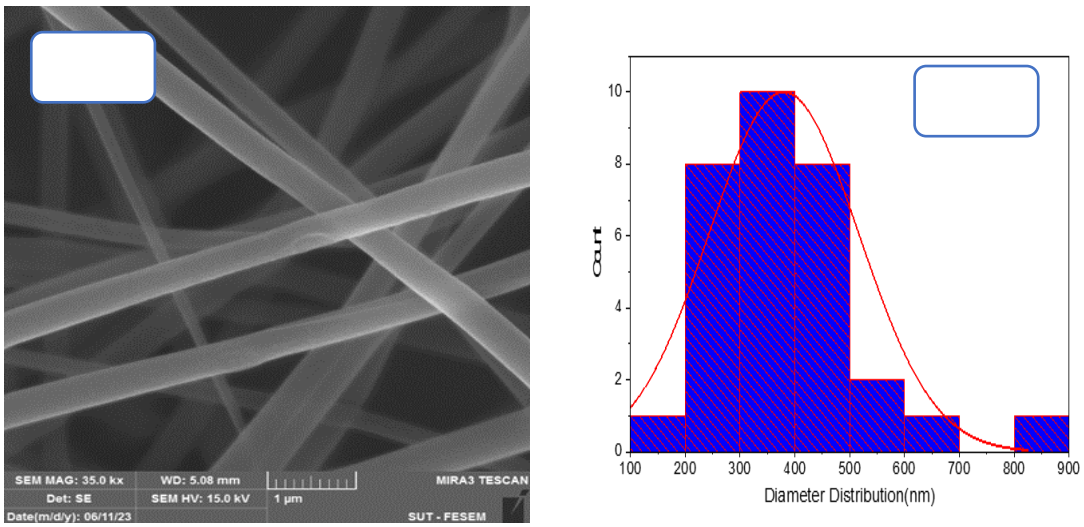

Fig.5: (A) FESEM Images With 35000 Magnification Powers , (B) Diameter Distribution Of TPU / Plavix Nano Fibers.

4.5 Atomic force microscopy (AFM) results

For sample TPU/Plavixwas showed in figure 4.6. The homogeneous small pores could promote initial osteoblast adhesive response Meanwhile, the arrangement and distribution of the pores in the Plavix were more regular and smaller, probably due to the comparatively low pH value of the Plavix[9]. increasing in the polymer concentration causes an decrease in scaffold porosity and the increase in scaffold density[13].

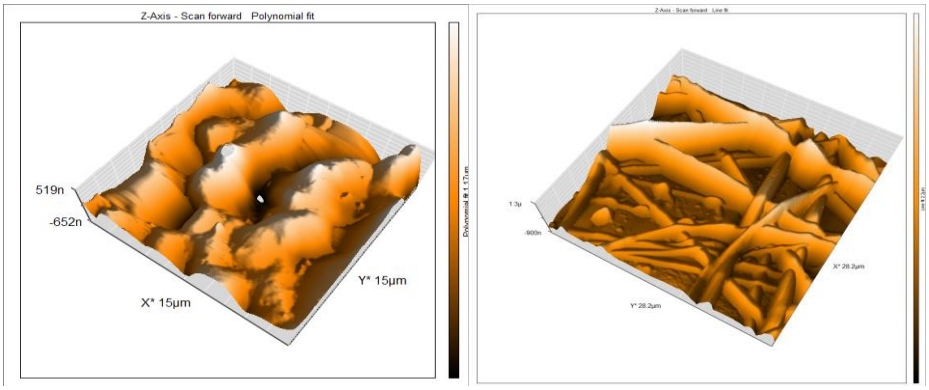

Fig 6: 3D- Micrographs AFM Images For TPU and TPU/Plavix Nanofibers

4.6 Microbial test

The antibacterial effects of the membrane was evaluated against Staph, and E. coli by detecting the growth inhibition of bacterial isolates around the membrane disks. The membrane was more effective against Staph followed by E. coli (Fig. 7). However, the value for neat TPU and TPU/Plavix –ve and 20 in Staph bacterial respectively. While in E. colibacterial is –ve and 16 for neat TPU and TPU/Plavix respectively.

Figure 7. The Antibacterial Effects Of The Membrane Against Staph And E. Coli By Detecting The Growth Inhibition Of Bacterial Isolates Around The Membrane Disks.

4.8 Hemocompatibility Assessment of The Scaffold Material

4.8.1 Activated partial thromboplastin time (APTT)

Blood coagulation and anticoagulant capabilities of pure TPU and TPU/Plavixnanofibers were studied using the activated partial thromboplastin time (APTT) and prothrombin time (PT). Both the extrinsic and intrinsic pathways were studied, with the PT focusing on the former and the APTT on the latter. Compared to neat TPU, the TPU/Plavix nanofiber produced in the current investigation showed a longer blood clotting time (APTT). Electrospun TPU/Plavixnanofiber was shown to have a blood clotting time of (115.6, 122.9, 126.1) s for three ratios (0.2, 0.6, 1) % from Plavix, whereas the TPU texture showed a duration of 103.8 s in the APTT experiment. When compared to TPU, the APTT value of the manufactured demonstrates that the nanofiber surface delays clotting time more effectively. APTT is a coagulation screening test that assesses the status of patients with coagulation abnormalities[14]. APTT was significantly higher than in sample TPU/Plavix, showing that Plavix could decrease the incidence of cardiovascular disease through alleviation of blood hypercoagulability and prevention of thrombosis[15].The PT assay showed similar results; the blood clotting time of the fabricated neat TPU was 28.2 s, while the blood clotting time of the electrospun TPU/Plavixnanofibers was 33.6, 36.5, and 38.1 s, as shown in Fig. 9. The sample made with 1% Plavix had a significantly longer APTT and PT value, indicating that the nanofiber surface is superior to TPU for enhancing anticoagulant nature [16]. The reduced fiber diameter observed by Milleret et al. will be helpful for blood compatibility. They fabricated scaffolds from two polymers, degarapol and poly (lactic-co-glycolic acid) (PLGA), and found that the smaller fiber diameter exhibited a delay in blood clotting, which appears to be in agreement with this findings [30].

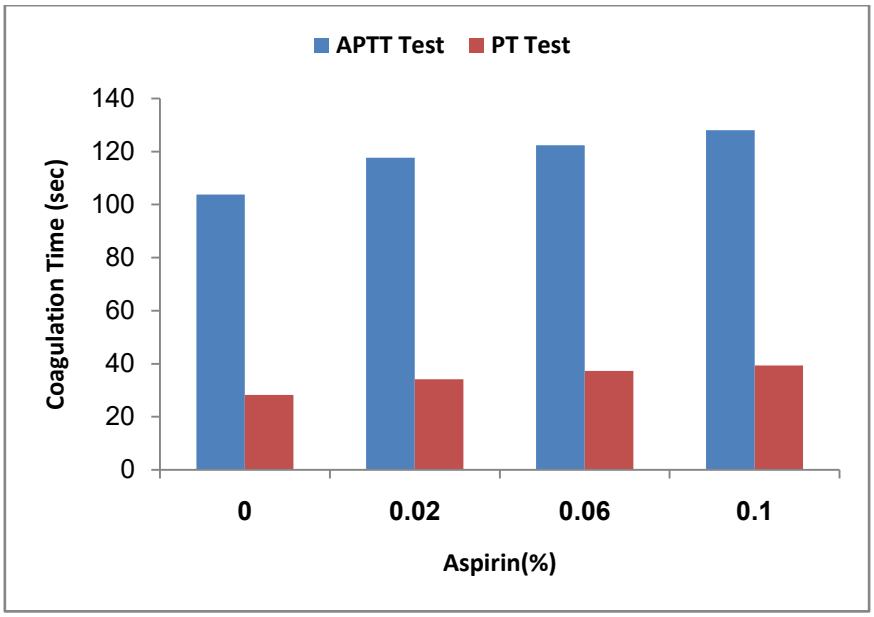

Fig. 9: (APTT and PT) Assay Of neat TPU and TPU/Plavix Nanofibers.

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