



## Nanotechnology In the Biomedical Field For Non-Woven Fiber Via Electrospinning Technique Embedded With ZnO / GO On Wound Healing (Cell Migration)

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### Abstract

**Introduction:** Chronic wounds represent a significant public health challenge, particularly for older individuals with comorbidities. This research aims to explore the potential of nanotechnology developments in the biomedical field, specifically investigating the use of polyurethane nanofiber embedded with a combination of Zinc Oxide (ZnO) and Graphene Oxide (GO) for wound healing applications. **Methods:** The study utilized the electrospinning method to fabricate the polyurethane nanofibers and a green synthesis approach of zinc oxide. The wound healing properties of the ZnO-GO-embedded polyurethane nanofibers were evaluated through a series of characterization techniques, including cell migration assays, scanning electron microscopy (SEM), Transmission Electron microscope (TEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). **Conclusion:** The findings of this research suggest that the utilization of polyurethane nanofibers could be a viable approach for addressing the challenges associated with chronic wound management.

**Keywords:** Zinc Oxide (ZnO), Graphene Oxide (GO), Electrospinning, Cell migration, Wound healing.

### Introduction

Currently, Nanotechnology has been developed including nanofibers with applications in many fields. Nanofibers can be fabricated via the electrospinning method, as this method results in nanofibers with a small diameter on the nanoscale. Applications of nanofibers in the biomedical field include using nanofibers to accelerate the wound-healing process on the skin of the human body. [1,5,6]

Recently, the biomedical field has witnessed such great development, especially in wounds that don't heal well, the skin is the most important and largest organ in the human body, and its primary function is protecting the body. [12] Since wounds are the most important problem that the skin faces, the speed of their healing is important. Also, Chronic wounds represent an obstacle to public health, and it is expected to increase due to their resistance to antibiotics. Furthermore, it was the reason for more future studies to solve this problem. In these regards, nanomaterials got great attention because of their properties. [7,8,11]

The electrospinning method is one of the fastest-growing ways that uses polymer-based nanofiber fabrication. Electrospinning is a technique that produces fibers in nanometer size, by the electric current of a high voltage potential between two electrodes of opposite polarities. It can overcome the surface tension of the solution, or melt, allowing the formation structure of the fiber and the solvent evaporation. This technique has wide use in biomedical fields such as non-woven fabric embedded with additives, wound healing, tissue engineering, and tissue for regenerative

medicine.[10] The used polymer must have good mechanical, chemical, and temperature stability, electrical conductivity, biocompatibility, and electrical activity. Here we use polyurethane which has excellent physical properties, biocompatibility, chemical resistance, and biodegradability. [1,2,14,]

In this work we have extracted zinc oxide in a green way from orange fruit peels to reduce using toxic chemicals and be safer, and for its properties. Electrospun fibers can adjust their properties to meet specific requirements such as stability, surface area,

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and mechanical strength. by controlling the process parameters such as additives, polymer composition, and solvents. Antibacterials, additives, antimicrobials, and surfactants are doped into the electrospinning process by either loading the additive in solution before performing electrospinning, co-spinning material with an additional independent needle, or by post-processing a previously electrospun sample. Additives can help optimize the solution processability, enhance the sample material characteristics (rheological properties, morphology, chemical resistance), or even allow new material functionality (surface chemistry, reactivity). [1,3,4]

## Materials and Methods

### Materials

Zinc nitrate salt (Sigma-Aldrich, crystallized 99.0%, USA), (Orange peel) for its high content of organic compounds, Graphene oxide (Sigma-Aldrich, 2 mg/mL, USA), Poly Urethane (BASF Elastollan, TPU LP9175, made in German), Distilled water as the synthesis medium.

### Methods

#### Preparation of Orange peel extracts

The extracts were obtained by thoroughly cleaning and drying orange fruits before thinly peeling them. Afterward, the peel underwent a 12-hour drying process in a food drier until it became completely dry then, it was carefully ground into a powder with a smooth texture. 1 g of the powder was added to individual glass containers, each filled with 50 mL of distilled water. The mixture was stirred for 3 hours. The mixtures were subjected for 60 minutes to a water bath at 60 °C after being macerated. The combinations were filtered, and the resulting extracts were stored in an argon environment for future use. [4,19]

#### Preparation of Zinc Oxide nanoparticles

The liquid form of the orange peel extract was separated using a centrifuge at 6000 rpm. The outcome of this process is a top-notch extract that can be utilized for future experiments. In the process of synthesizing ZnO NPs, 15 ml of extract was gradually added to a solution containing 35 ml of Zinc nitrate salt (7 mM). The colloidal reaction was heated at 60 °C for 4 hours, with continuous stirring at 500 rpm. The ZnONPs underwent a noticeable change in hue, transitioning from colorless to brown. Following a 4-hour reaction phase, the resulting creamy-colored ZnONPs were carefully collected, cleaned, and subjected to centrifugation at 10,000 rpm. This process was repeated three times to ensure thorough purification. The nanoparticles were dehydrated using a vacuum machine in the end. [4,15]

#### Graphene Oxide loaded ZnO nanoparticles

The surface of GO was decorated with ZnO NPs using the previously published techniques with a slight modification.[22] The ZnO NPs (1 mg/ml) were evenly dispersed in 10 ml of de-ionized water and subjected to sonication for 10 minutes to achieve a homogeneous solution. Afterward, the Graphene Oxide was diluted to a concentration of 1mg/mL in 20 mL of de-ionized water and underwent sonication for 10 min in a water bath. The ZnO NPs solution was carefully added to the GO solution while gently stirring and then heated at 50 °C for 3 hours. The suspension color undergoes a noticeable transition from a dark yellowish shade to a deep black hue when visually observed. The ZnO/GO composite underwent centrifugation at 12,000 rpm for 12 min. The process was repeated three times to wash the material. Then, the composite was placed in an ice bath for 6 hours and then dried using a vacuum pump.[23]

#### Preparation of TPU\ZnO@GO NPs nanofibers

The nanofibers of TPU mixing were fabricated via an electrospinning technique [NANON-01, Japan]. Here is Table (1). The TPU pellets were dissolved in a solution of Di Methyl Formamide for 6 hours, resulting in a clear and viscous solution. No heating was required for this process. According to Table (1), 10 mL of TPU was added to a glass syringe with a capacity of 20 mL (equipped with an 18G needle), along with different substances derived from ZnO@GO NPs. A high-voltage generator with a power output of 30 kv was used. The direct current generator's positive terminal was connected directly to a collector device, which featured a rotating plate covered in aluminum foil. Figure (2) shows the nanofiber webs were fabricated with meticulous precision using specific parameters: injection of 3 mL/h in a flow rate, an applied voltage of 16 kV, and an electrical charging distance of 12 cm. The humidity and temperature were considered within the electrospinning device. [20,21]

### Characterization

#### Scanning Electron Microscope - SEM

Fiber structure was studied using device model DSM-965, the fiber was gold coated with a sputter coater (Blazers, SCD50). Diameters of Fiber were measured with the help of software. In every experiment, fiber diameter average, and allocation were decided from about 100 random measurements using micrographs that represent fiber morphology.

#### Transmission electron microscope- TEM

(TEM) was used to analyze the size, and crystalline structure, by JEOL (TEM-1230, Japan) device.

#### FTIR

Results were listed by a spectrometer (Nicolet-470Nexus). The FTIR spectrometer was purified constantly with nitrogen. In transmission mode, the

infrared spectra were recorded using stout films of spun (solution blow spinning and electrospinning) nanofibers polymer which were represented on a silicon flake.

### XRD

For dimensions, nonwoven Fibers were collected on aluminum foil and were stabilized on glass slides for analysis. Scans were done from the angle range (10-40) ( $2\theta$ ) at a scan rate of two min using  $\text{CuK}\alpha$  (Ni-filtered).

### Cytotoxic effect on human cell lines

Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan.[9]

### Scratch assay (wound healing assay):

migration of the cells was evaluated through a wound scratch assay. For the HSF cell line, we seeded  $5 \times 10^5$  cells/well in a 6-well plate and incubated them overnight at  $37^\circ\text{C}$  under a 5 %  $\text{CO}_2$  atmosphere. Next, removed the medium, scratched the adherent sheets of cells with a sterile 10  $\mu\text{l}$  pipette tip, and washed them with PBS. Finally, we added 3 ml of treatment low serum media (1% FBS DMEM) and treated the cells with the drug at doses corresponding to each compound's IC 30. [24,25]

### Results and Discussion

#### Scanning Electron Microscope (SEM)

Table 2 shows Figures (a,b,c,d) the structure of the nanofibers. The Polyurethane nanofiber has a regular and continuous morphology [1]. It has been noticed that the production of nanofiber increased when we used ZnO/GO with the polymer solution. Using graphene Oxide in the solution increases conductivity and reduces viscosity, so speeds up the jetting of the polymer during the electrospinning process.

#### Transmission electron microscope- TEM

Figure (3) shows the size of Zinc Oxide nanoparticles in the range of (10-20 nm).

#### FTIR

Figure (4) shows the PU sample. NH stretching matches the absorption band at  $(3323) \text{ cm}^{-1}$ . The peaks at  $(2859-2938) \text{ cm}^{-1}$  are linked with stretching  $-\text{CH}_2$ , and other bands of  $-\text{CH}_2$  vibrations are identified at  $(1464-1418-1364-1294) \text{ cm}^{-1}$ . Further, a C=O group in polyurethane is linked with the absorption band at  $(1734) \text{ cm}^{-1}$ . Bands of NH group vibrations are identified at  $(1541) \text{ cm}^{-1}$ . hydrogen bonding between N-H and C=O groups at  $(1702) \text{ cm}^{-1}$  is assigned to the (hard/ester) segment or ester-oxygen groups of the soft segments of urethane bonds. nonhydrogen-bonded carbonyl groups band at  $(1720) \text{ cm}^{-1}$ . [1,26]

Figure (5) shows that the Polyurethane structure is not affected by the presence of zinc oxide. Electrospun PU shows characteristic bands for (N-H), (C-H), (COO), and (C-C) bonds at

$(3,320-2,960-1,730-1,703-1,530-1,220) \text{ cm}^{-1}$ , respectively. the adsorption of ZnO appeared at the  $509 \text{ cm}^{-1}$  band. So, the result suggests the union of ZnO in the combined nanofiber.

Figure (6) shows the wavelength for the original O-H  $(3300-3600) \text{ cm}^{-1}$  the sample becomes more visible because the absorption peak, such as wavelength for C = O at  $(1670-1780) \text{ cm}^{-1}$ , C = C  $(1650-1675) \text{ cm}^{-1}$ , C-O at  $(1050-1150) \text{ cm}^{-1}$ , also naturally appeared absorption peaks coupled with some other. wherefore, the analysis of FTIR showed that we successfully converted the graphite into a high purity Graphene Oxide. [22,23]

### XRD

Figure (7) shows The XRD results, confirmed the presence of ZnO NPs in the dispersed combine. In the original polyurethane, the spectrum showed no peak, which is a reference to the polymer structure nature.[2]. While in PU nanofibers doped with the zinc, bands strongly appeared with maximum peaks at  $(31.2^\circ-35.8^\circ-38^\circ)$  as Bragg's reflection represented from the (100,101,110) planes, which means there are crystalline ZnO. Overall, there was an increase in the crystallinity of the PU polymer because of ZnO.

In figure (8) TPU@ZnO\GO nanofiber, Graphene Oxide sheets linked on the surface of nanofibers. like reports in the literature Polyurethane nanofiber film shows a peak around  $20^\circ$ . In the case of the GO/PU nanofiber membrane, the peak of Graphene Oxide was observed at  $2\theta$  value of  $(38^\circ)$ . More observations for peaks were at  $2\theta$  values of  $(31.2^\circ-34.3^\circ-36.4^\circ)$  identical to the crystal planes (111-200-220-311).

Further, GO was significantly reduced to RGO indicated by Graphene Oxide peaks indicating that it had been well integrated within the Fiber.

### Cytotoxic effect on human cell lines

All the following procedures were done in a sterile area using a Laminar flow biosafety cabinet Class II A2 (Manufactured by: Labconco). Cells were suspended in DMEM medium, 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000 $\mu\text{g/ml}$  Streptomycin Sulfate, and 25 $\mu\text{g/ml}$  Amphotericin B), and 1% L-glutamine and 5% fetal bovine serum at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$  using  $\text{CO}_2$  incubator (Sartorius stedium, biotech).[13]

Cells were batch cultured for 10 days, then seeded at a concentration of  $10 \times 10^3$  cells/well in fresh complete growth medium in 96-well plastic plates at  $37^\circ\text{C}$  for 24 h under 5%  $\text{CO}_2$  either alone (negative control) or with different concentrations of drugs to give a final concentration of (1000, 500, 250, 125,

62.5, 31.25, 15.625 ug/ml). After 48 h of incubation, the medium was aspirated, 20 ul MTT salt (2.5µg/ml) was added to each well and incubated for a further four hours at 37°C under 5% CO<sub>2</sub>. To stop the reaction and dissolve the formed crystals, 200µL of 10% Sodium dodecyl Sulphate (SDS) in 0.01M HCL was added to each well and incubated overnight at 37°C.[25]

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm.

Viability = absorbance of drug/absorbance of control x 100

Cytotoxicity = 100- viability. [16,17]

#### **Scratch assay (wound healing assay):**

We recorded cell migration into the wound space using an inverted microscope and optical camera (ZEISS ZEN microscope software blue edition) at 0, 24, and 48 after treatment.[17]

The distances of the cell migration were recorded and appeared in the figures.

The sample was tested against Normal Human Skin Fibroblast (HSF cell line), Sample concentration ranges between (1000 to 15.625 ug/ml) using MTT assay. [18,24]

#### **IC50:**

concentration of the sample causes the death of 50% of cells in 48 hours in Table (4).

As Table (5) shows the best results in samples embedded with zinc oxide and graphene oxide with concentration 0.1 and 0.2% [(TPU@ZN/GO 0.1%), (TPU@ZN/GO 0.2%) ]as the wound healed with rates of 51% and 50.7%, as the control sample of the pure cells did a self-healing without any additive with a rate of 8.2% and at concentration 0.3%(TPU@ZN/GO 0.3%) the wound affected by the concentration which has an opposite effect on the

healing rate 4.9%. the sample (TPU@ZN 0.5%) gives 26.3% of healing rate less than the samples with graphene oxide which means that graphene oxide has a healing effect.

#### **Conclusions**

In this research, Polymer materials can be manufactured into wound dressings, namely Poly urethane (TPU), combined with other additives like zinc oxide extracted in a green way and graphene oxide via electrospinning method that helps wound healing on the skin tissue, However, graphene-based materials have shown great potential in accelerating the healing process of infected wounds other than zinc oxide only and we hear more about them in near future. The global wound care market size was valued at USD 20.8 billion in 2022 and is expected to expand at a compound annual growth rate (CAGR) of 5.4% from 2022 to 2027 (USD 27.2 billion). This will encourage academic as well as pharmaceutical and medical device industries to investigate any new materials and products for the treatment of wound healing. Structural properties of TPU@ZnO/GO nanofiber and nanomaterials were characterized by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron microscope (TEM) and Fourier Transform Infrared Spectroscopy (FTIR), showed a proven successful result. The composition of ZNO and GO with Polyurethane nanofibers via a one-step electrospinning process was successfully proved.

#### *Acknowledgment*

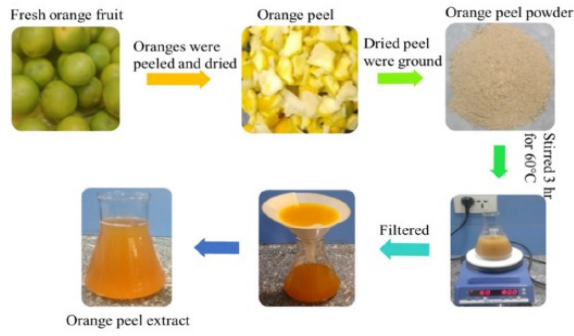
The authors appreciate the input from the anonymous reviewers that greatly improved this research study.

#### *Conflicts of interest*

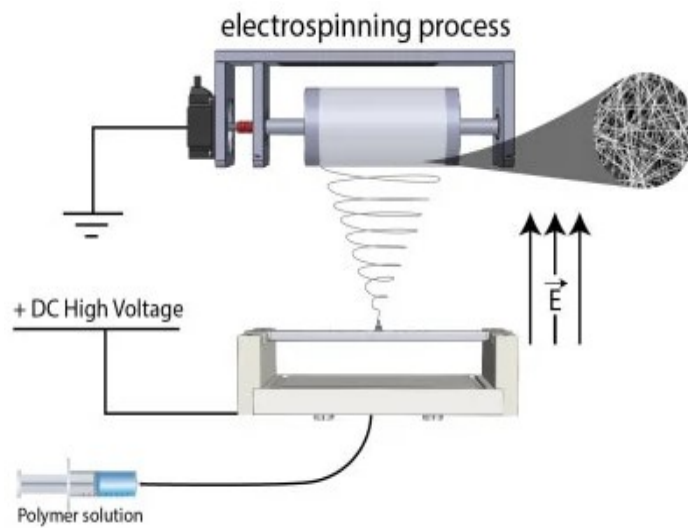
There is no conflict of interest in the publication of this article.

#### *Funding statement*

None.



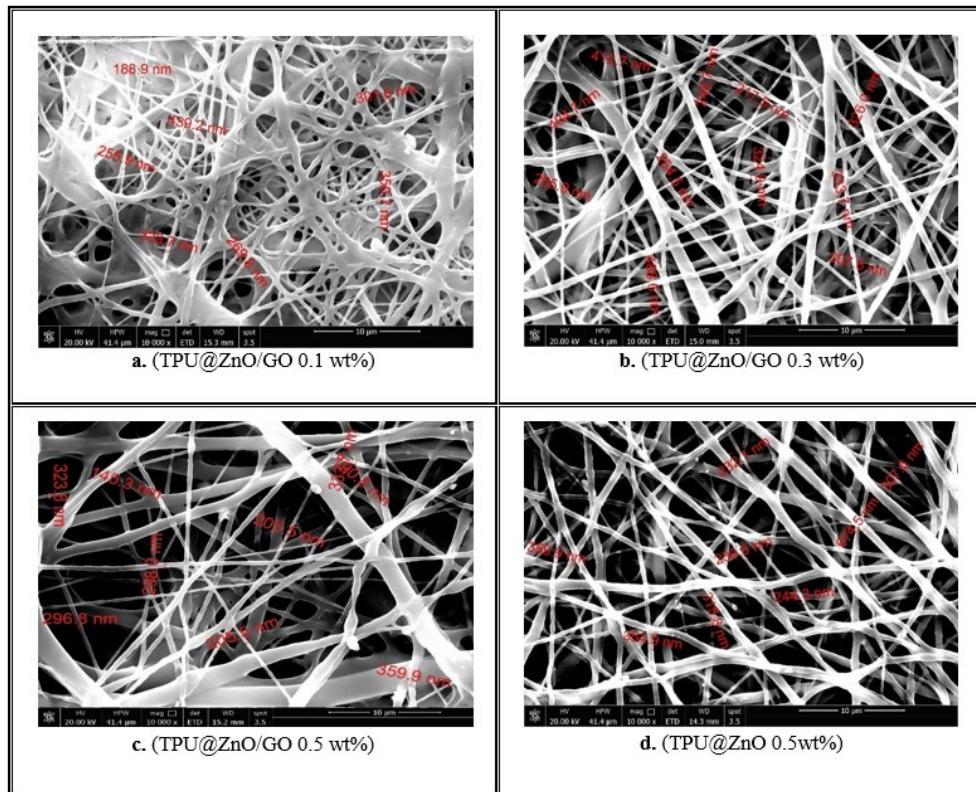
**Fig 1. Preparation of green synthesis of orange peel extracts**



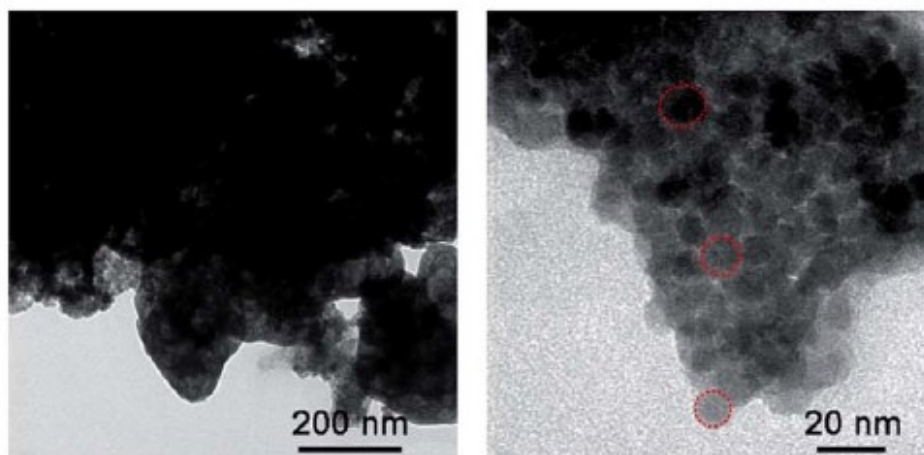
**Fig 2. Electrospinning process**

**Table 1. Designed samples and ZnO/GO NPs concentrations.**

Code	TPU conc. (Wt.%)	ZnO@GONPs (Wt.%)
TPU	10.0	0
TPU\ ZnO	10.0	0.5wt.%
TPU\ZnO@GONPs (0.1wt. %)	10.0	0.1wt.%
TPU\ZnO@GONPs (0.3wt. %)	10.0	0.3wt.%
TPU\ZnO@GONPs (0.5wt. %)	10.0	0.5wt.%



**Table 2:** SEM of nanofibers with different concentrations of additives



**Fig 3.** TEM for Zinc Oxide nanoparticles.

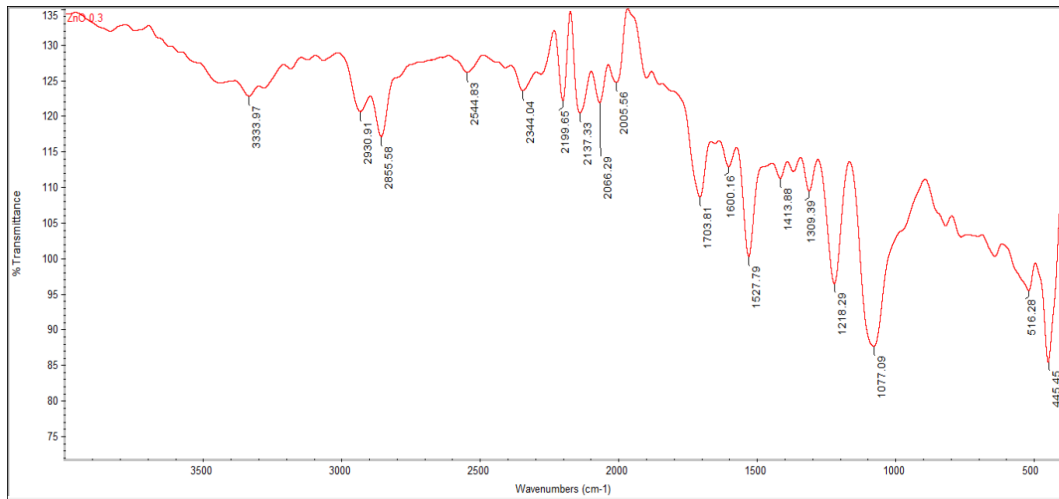


Fig 4. FTIR for Polyurethane (PUA 1,2,3: different polyurethane samples show convergent results).

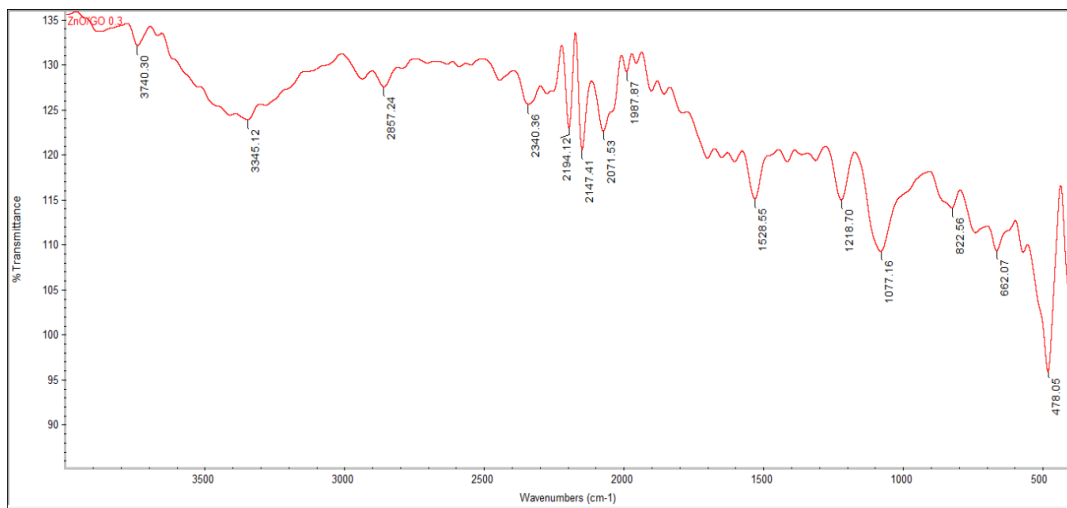


Fig 5. FTIR for TPU@ZnO

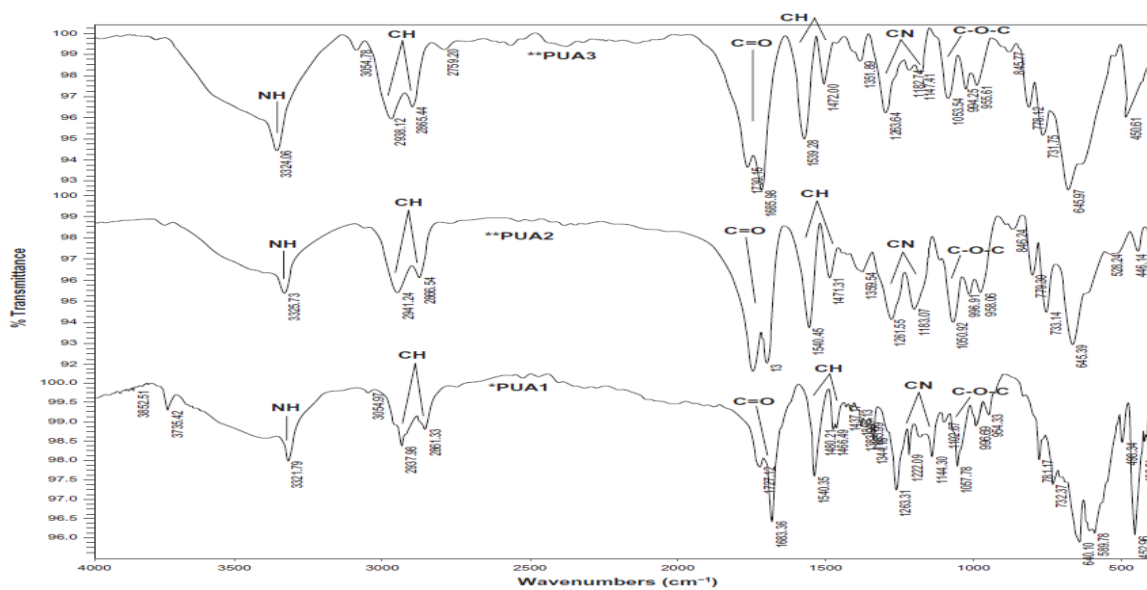


Fig 6. FTIR for TPU@ZnO/GO

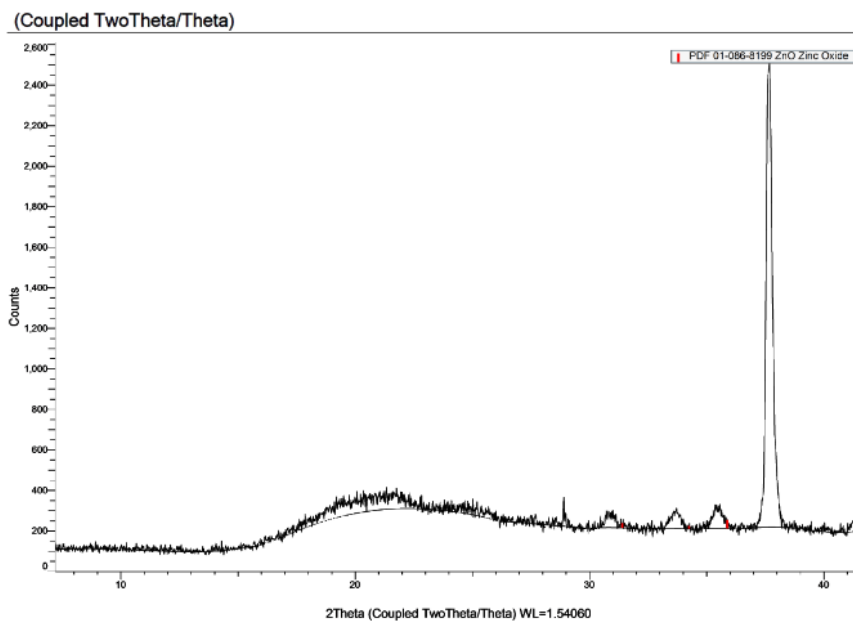


Fig 7. XRD for TPU@ ZnO

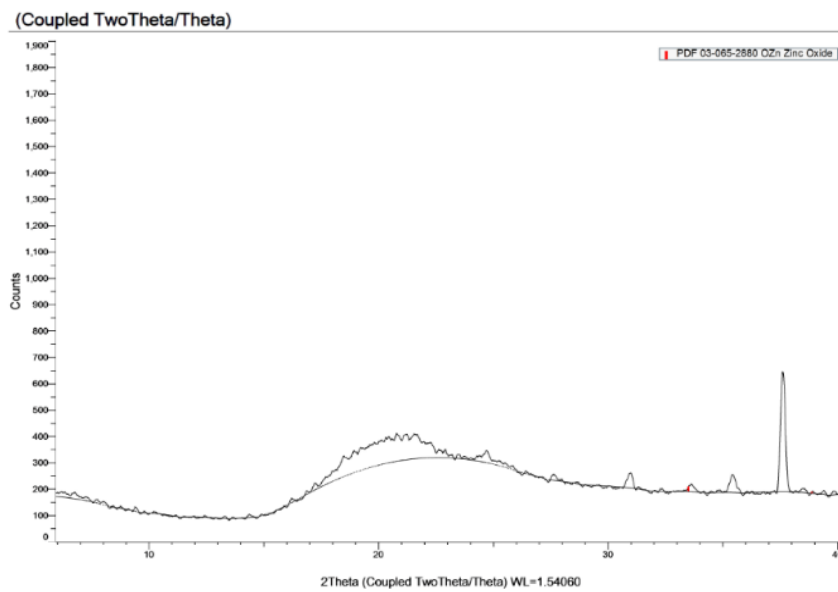


Fig 8. XRD for TPU@ZnO\GO

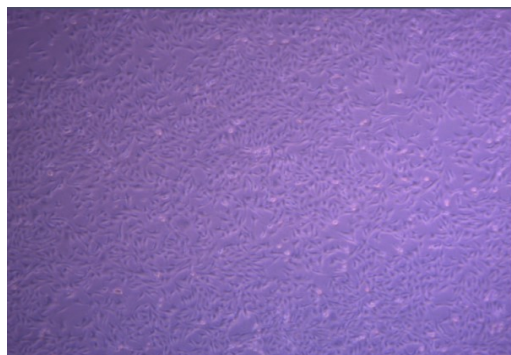
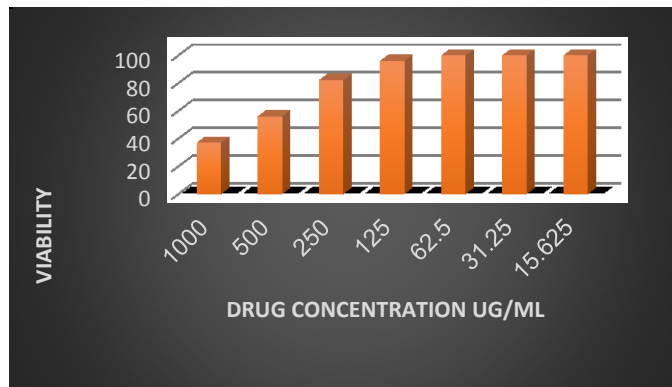


Fig 9. HSF cells



**Table 3. Cytotoxicity of different concentrations of drug**

Different dilution of Drug (ug /ml)	Viability %	Cytotoxicity %
1000	37	63
500	56.2	43.8
250	82.2	17.8
125	96.1	3.9
62.5	100	00
31.25	100	00
15.625	100	00



**Fig 10. Cytotoxicity of different concentrations of drug**

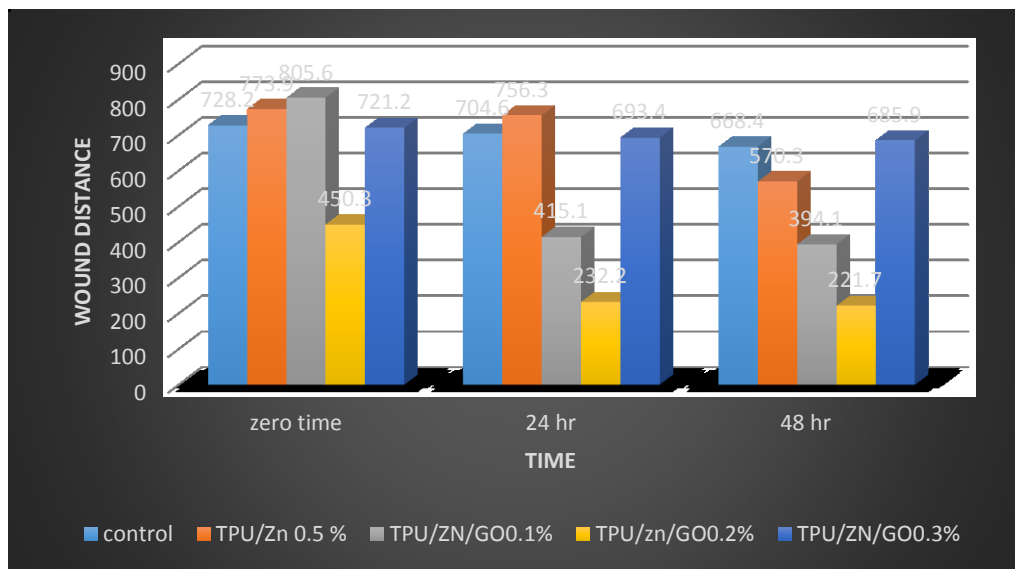
**Table 4. Concentrations of IC**

IC 50	721.2503	ug/ml
IC 30	444.5571	ug/ml
IC 90	1274.637	ug/ml

Concentrations	Zero time	After 24hr	After 48hr	Percentage of healing
Control (cells self-healing)				8.2%
TPU@ZnO 0.5wt%				26.3%
TPU@ZnO/GO 0.1 wt%				51%

<p><b>TPU@ZnO/GO 0.3 wt%</b></p>				<p><b>50.7%</b></p>
<p><b>TPU@ZnO/GO 0.5 wt%</b></p>				<p><b>4.9%</b></p>

**Table 5:** Wound healing after (0,24,48) hr



**Fig 11.** Wound healing after (0,24,48) hr

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## تقنية النانو في المجال الطبي الحيوي للألياف غير المنسوجة عبر تقنية الغزل الكهربائي المضاف إليها أكسيد الزنك وأكسيد الجرافين في التمام الجروح (هجرة الخلايا)

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<sup>2</sup> المعهد العالي بالتجمع الخامس- فنون تطبيقية- قسم طباعة المنسوجات والصباغة والتجهيز-القاهرة - مصر.

### المستخلص

مقدمة: تمثل الجروح المزمنة تحديًا كبيرًا في الوقت الحالي على الصحة العامة، وخاصة بالنسبة للأفراد الأكبر سناً الذين يعانون من أمراض مزمنة ومصاحبة للسن الكبير. يهدف هذا البحث إلى استكشاف إمكانات تطورات تكنولوجيا النانو في مجال الطب الحيوي، وتحديدًا دراسة استخدام ألياف البولي يوريثان النانوية المدمجة مع مزيج من أكسيد الزنك (ZnO) وأكسيد الجرافين (GO) لتطبيقات التمام الجروح. الطرق: استخدمت الدراسة طريقة الغزل الكهربائي لتصنيع ألياف البولي يوريثان النانوية وطريقة التوليف الأخضر صديق للبيئة لأكسيد الزنك. تم تقييم خصائص التمام الجروح لألياف البولي يوريثان النانوية المضمنة بـ ZnO-GO من خلال سلسلة من تقنيات التوصيف، بما في ذلك فحوصات هجرة الخلايا، والمجهر الإلكتروني الماسح (SEM)، والتحليل الطيفي للأشعة تحت الحمراء لتحويل فورييه (FTIR)، المجهر الإلكتروني النافذ (TEM). وحيود الأشعة السينية (XRD). الخلاصة: تشير نتائج هذا البحث إلى أن استخدام ألياف البولي يوريثان النانوية يمكن أن يكون طريقة قابلة للتطبيق لمواجهة التحديات المرتبطة بشفاء الجروح المزمنة تم بنجاح إثبات تركيب ZnO و GO مع ألياف البولي يوريثان النانوية من خلال عملية الغزل الكهربائي بخطوة واحدة.

**الكلمات المفتاحية:** أكسيد الزنك (ZnO)، أكسيد الجرافين (GO)، الغزل الكهربائي، هجرة الخلايا، التمام الجروح.