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Bioactive Composite Nonwoven Surgical Dressing based on Cellulose Coated with Nanofiber Membrane using the layer-by-layer technique

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Abstract

Nonwoven fabric with a cellulosic base has many features, including the ability to absorb biological fluids and can easily adapt to different types and locations of wounds, which makes it common and unique in surgical dressing. The study aimed to design different samples made of one layer and two layers of nonwoven fabrics for the inner layer of deep endoscopic wound dressings to control the biological fluid leakage. 100% cotton, 100% viscose, and viscose/polyester (70:30) were used as one-layer samples, and the two different materials were used interchangeably to design the two-layer (layer-by-layer) samples. One-layer samples were dyed using green coffee extraction, while the two-layer samples were enhanced by adding powdered green coffee between them, as an innovative use in the surgical dressing applications. An electrospinning technique was used to form a delicate membrane of Ciprofloxacin (CIP) antibiotic and Kylie Collagen to coat the single-layer samples without coffee dyeing to compare their performance with the dyed samples. Also, to compare the results of the two layers of samples that were enhanced with green coffee powder with and without the coating nanofibers membrane, Designed samples were evaluated for mechanical and physical properties for nonwoven fabrics, in addition to FTIR, SEM, absorbency and antimicrobial activity, and K/S, especially for dyed samples, and finally cytotoxicity for CIP, Kylie collagen, and green coffee. The results pointed to the fact that the dyed one-layer samples with green coffee extraction achieved a high-performance function for air permeability and antimicrobial activity, while the layer-by-layer samples that were enhanced by adding green coffee powder showed improvement for strength, absorbency properties, and antimicrobial activity, especially for the samples made of cotton/viscose layers and viscose/viscose blended polyester layers, followed by the sample of cotton/viscose blended polyester layers that were coated with 0.1wt% CIP antibiotic and the Kylie Collagen membrane.

Keywords: Layer-by-Layer, Electrospinning, CIP Antibiotic, Collagen**,** Green Coffee.

1. Introduction

Wound healing is a correlation between cytokines that promote the growth of membranes and tissues, prevent dehydration and increased inflammation, growth factors, blood, and extracellular matrix. The function of these elements is also affected by the local environmental factors like temperature, oxygen percentage, exposure to infection, the patient's health status, and sometimes the nature of the patient's skin. In addition to how to care for and treat wounds,[1-3]. Medical wound dressings play a big role in the healing process either directly or indirectly by removing the necrosis, getting rid of dead tissues, as well as cleaning and sterilization of wounds to prevent infection and promote the growth of new cells [1, 4]. The types of dressings and the usage methods differ according to the nature of the wounds and their causesThe types of dressings and the usage methods differ according to the nature of the wounds and their causes [5-8]. The type of wound dressing materials must be suitable for medical use and not cause cancer or skin allergies when they come in contact with the patient's skin [7, 9, 10].

Nonwoven fabric is considered a suitable technique for wound dressing largely; because it reduces the possibility of contamination from the air and provides comfort sense, it affords an

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effective barrier against bacteria, especially when adding materials that increase this effectiveness [11, 12]. Composite dressings are multiuse and acceptable for partial/full areas of wounds but have less flexible and are more expensive. Composite dressings consist of multiple layers each layer is physiologically distinct. Most of the composite dressings are formed from three layers. It may include adhesive edges of nonwoven fabric. The outer layer protects the wound from infection, the middle layer is composed of an absorptive material that maintains the moisture and assists autolytic debridement, and the inner layer is composed of non-adherent material to prevent from sticking to young and new granulating tissues of skin [1, 3, 7].

Cellulose based fabrics are the most common material in wound care uses for its high absorption capacity and tolerance of washing and sterilization processes. So, several studies concerned with treating cotton to provide bacterial resistance, Mary Grace *et al*. reached to that the copper nanoparticles loaded cellulose fibers that gave excellent antibacterial against *E. Coli*, they have great potential to be used as dressing materials [13-16].

Viscose fibres regenerated cellulose produced from cotton Linters [17]. Viscose gives a soft and silky feel, its fibers do not shrink with heating, it has the aptitude to respire, biodegradable, thermal stability, and has good drapability property, but it creases easily when immersing in hot water. Viscose can be blend with other fibres like polyester, and cotton. These blends are commonly used for medicinal purposes to offer absorption and comfort properties [18, 19]. A. Abou-Okeil *et al*.2012 reached to that the usage of nonwoven fabrics from 100% viscose and viscose blended with polyester that treated with chitosan/PVA/Ag nanoparticles helped to heal wounds and give an adequate air permeability rate and suitable absorbing of wound fluids after performing the surgical experiment on French White Bouscat rabbits [20, 21].

Coffee was one of the beans used at ancient Chinese, Egyptian, and Desert Arabs ages to treat wounds, aging, and poor memory. Chemical constituents of coffee are fairly fixed; phenolic and its derivatives, alkaloids (especially caffeine), terpenoid, carbohydrate, lipid, volatile, and heterocyclic compounds as mentioned by Brezová et al., 2009 [22]. Recently, the researchers reached drinking green coffee increases mental alertness and concentration, reduces death' risks of cancer and heart disease especially for women, and blood pressure due to Chlorogenic acids (CGA) in green coffee bean extract (GCE) [23, 24]. There are two commercial types of coffee; green & black coffee, black coffee is made from green coffee after the addition of a flavor and color [25, 26].

Green coffee oil is considered a high concentration of essential fatty acids (Hydroxycinnamic acids) such as Caffeic and Ferulic acid, mainly in a form of mono and diesters with quinic acid referred to as chlorogenic acids (CHAs) that have a broad range of biological activities such as antibacterial, antifungal, hepatoprotective, antithrombotic, anti-inflammatory and antioxidant activities that decrease the risk of several oxidative stress-related diseases, including atherosclerosis, some kinds of cancer and Alzheimer's disease . Also, green coffee has sterols that promote excellent moisture retention, quick penetration, and good adherence within cosmetic applications. In addition to, vitamin E makes it a potent antioxidant against the prevention of the development of photo-aging, inflammatory skin disorders as eczema, psoriasis, acne-prone, and problematic skin. in addition to skin damage by sunburn cell formation and DNA degradation [27-29]. Overall, Robusta coffee bean extract 45% ointment can give a good effect on the wound healing process, as at this concentration the number of fibroblasts increased significantly as mentioned by Cavia cobaya [30]. The topical treatment with green coffee oils led to cells' normal actions and faster wound healing in rats [31]. Other researches showed the effect of green coffee extract in the rejuvenation of aging skin, which was revealed by the increase of collagen and RNA level in rat's skin [32]. Also, Humaryanto, Ave OR concluded that the usage of green coffee extraction beans that have Ethanol 70% accelerated the healing of rats' wounds as 1:3 compared to those that did not use this extract [33].

Wound healing is a complex process in which vital collagen plays a major role. Thus, it is considered the structural scaffold for the proliferation of new cells during wounds, due to its ability to attract the new fibroblast and keratinocytes cells that make the skin look natural after healing, in addition to muscle fibroblasts that contract the wound area during stages of healing [3, 33].

In the past few years, a five-year-old child was severely burned from a boiling water spill, the treatment was done at home by using and rudimentary green coffee powder, the child's skin was completely healed within three weeks. So, the Drug Information Center at Howard University (HU), raised the attention to the need to search for active compounds in green coffee powder [34].

The present research study aimed to the preparation of bioactive composite nonwoven as an inner layer (contact with skin) of wound dressing for endoscopic surgery from cotton, viscose, and viscose/ polyester materials; using dyeing with green coffee extraction for one layer samples and green coffee powder to enhance the layer-by-layer

dressing, to utilize green coffee medicinal effect with different new manner, and improve the medical performance of the designed samples. CIP antibiotics and Kylie Collagen have also been used as a nanofiber coating membrane by electrospinning technique for some designed samples to achieve both higher antibacterial activity and non-toxic bioactive materials for biomedical textiles.

2. Materials and Methods

2.1. Materials

2.1.1. Fabric Samples

Viscose/polyester (70:30)% spun-bond nonwoven fabric, 100% cotton, and 100% viscose spun lace nonwoven fabrics were used in this study. Table 1 presents the characterizations of the three main nonwoven fabrics used.

Table 1. Characterizations of the nonwoven fabrics.

Sample	Weight (g/m^2)	Thickness (mm)	Air Permeability ($cm^3/cm^2.s$)	Stiffness (mLg)	Burst Strength (Kpa)	Water Absorbency (Sec.)
100 % Cotton	35	0.304	271.5	658.6	155.6	0.623
100% Viscose	100	0.539	111.3	1530.8	295.7	0.737
Viscose/ Polyester (70:30)%	80	0.544	222.6	1673	311.3	0.38

2.1.2. Chemicals

Green Coffee Powder and Alum were purchased from local markets. Ciprofloxacin (CIP) antibiotic was kindly supplied from Memphis pharma and chemical industry, Collagen powder, Bovine species, calfskin source with chain composition C3511 provided from Sigma Aldrich, USA.

2.2. Methods

2.2.1. Dyeing Procedure

The dyeing process with green coffee for the one-layer of nonwoven fabrics was carried out in four stages as follows:

2.2.1.1. Extraction of dyes from green coffee

Adding 20g commercially available powdered green coffee to 100 mL distilled water prepared aqueous extract of green coffee. The mixture was stirred, heated, and hold at a boil for 30 min, allowed to stand for 15 min and then filtered. The filtration was used for dyeing.

2.2.1.2. Pre-treatment by scouring /boiling off:

An alkaline pre-treatment in a water solution containing sodium hydroxide (NaOH) 4.0 % (o.w.f) and wetting agent – Triton X- using liquor ratio 1:50 at boiling for 90 minutes. Then rinsing with hot and cold water then air-dried at room temperature. The purpose of this process is to get rid of foreign substances like lignin and wax…etc., whether natural or added, and boiling them in a solution of caustic soda to turn them into simple materials that can easily be removed with water.

2.2.1.3. Mordanting (fixing dye with fiber);

Mordants are auxiliary dyeing substances that can form complexes with molecules of dyes. This leads to an increase in the exhaustion or low-affine dye from the bath and thus improving the colour fastness. This particularly improves the wet fastness and sometimes the lightfastness. Alum is a traditional natural inorganic mordant that is hydrated with double sulfate salts. It is often called the hydrated aluminum potassium sulfate ($KAl(SO_4)_2 .12 H_2O$) [35-37]. So, the dyeing in presence of Alum fixes the components extracted from green coffee on the fabric at the dyeing bath.

2.2.1.4. Dyeing;

The scoured samples were dyed with dye extract from green coffee keeping an M:L ratio of 1:20, 1% Alum, 5% extraction green coffee (w.o.f). The dyeing was carried out on a Microwave for 3 min. After dyeing, the dyed samples were washed with cold water and dried at room temperature.

2.2.2. Electrospinning Technique process

2.2.2.1. Electrospinning Solutions

Polymer solutions used for electrospinning were prepared as follow: polyvinyl alcohol (PVA) solution (10 wt. %), was prepared by dissolving 1 g of PVA, containing ciprofloxacin antibiotic CIP (0.1 g) and Kylie Collagen (100 µL), in 10 mL distilled water under with moderate stirring at 60^o C for 2 h. [38]. The solution was prepared again in the same ratio for all components with an increase in the ratio of CIP antibiotic to be (0.4g).

2.2.2.2. Electrospinning Nanofibers

The viscous solution of PVA/CIP/collagen composites was contained in a plastic syringe. The pinhead was connected to a high-voltage generator, an aluminum foil served as the counter electrode. A dense web of fibers was collected on the wound dressing placed on top of aluminum foil. The utilized electrical potential amounted to 25 kV, the distance between the capillary and the substrate electrode was 15 cm, and the feed rate of the solution was 1 mL/h through a syringe pump. The electrospinning was performed at room temperature

and air humidity at an open system. The electrospinning device used consists mainly of an extrusion system (syringe pump), collecting electrode, and high voltage supply. Samples from nanofiber coatings with aluminum foil were taken for SEM imaging and samples from nanofiber webs on gauze bandage were taken for antibacterial evaluation [38].

2.2.3. Samples Preparation

Twenty-four samples were prepared and divided into two main groups according to the number of layers; whereas the samples of the first group formed from one layer of the nonwoven fabric, while the samples of the second group formed from two layers of nonwoven fabrics. Each main group consists of twelve samples, divided into four subgroups based on different modification methods (dyeing for one layer samples, enhancing with effective materials for layer-by-layer samples, and electrospun nanofibers coating) and effective materials (green coffee, collagen, and antibiotic) applied on the samples; whereas the sub-groups 1^A &2^A have the blank samples that formed from either one layer or two layers, respectively, see Table 2.

The sub-groups 1^B &2^B that modified with green coffee only; 1^B group have the dyed one-layer samples with green coffee extraction, as it is difficult to fix powdered green coffee on the one-layer samples. While the 2^B group represents the designed two-layers (layer-by-layer) samples that were enhanced by adding green coffee powder between them.

The sub-groups 1^C &2^C point to the samples designed from one layer without dyeing and layer-by-layer samples enhanced with powdered green coffee, respectively those coated with nanofibers membrane of 0.4g Ciprofloxacin antibiotic/100mL PVA and 5mL Kylie Collagen by electrospinning technique.

Finally, the sub-groups 1^D &2^D that point to the samples designed from one-layer without dyeing and layer-by-layer samples enhanced with powdered green coffee, respectively, those coated with fibers web of 0.1g Ciprofloxacin antibiotic/100mL PVA and 5mL Kylie Collagen by electrospinning technique.

Each research sample was designed with a size of (25×25) cm, three separate samples were made for each design with the same parameters to verify the sample results. The samples of two layers were fixed by an adhesive sheet from low melting point polyester weighed about 15 g/m^2, where the cohesion is made by pressure with heat. The weight of designed samples depended on the weight (g/m^2) of each sub-layer of nonwoven fabric materials (see Table 1), and the weight of the adhesive sheet, and the amount of powdered green coffee that about (33-36) g/m^2 for each sample. In the case of the dyed samples with the extraction of green coffee for one- layer the ratio was explained in dyeing possess.

Samples that have proven effective performance will be used as an internal layer in contact with the patient's skin (disposable layer), they will be prepared to study the biological effects in the following study.

The sub-groups 1^C &1^D samples are represented in Fig.s 1 and 3, and the 2^C &2^D samples are in Fig.s 2 and 3. Each sub-group contains three samples according to the different materials nonwoven fabrics. Table 2 represents the designed samples code and their description that will be used in the following fig.s and tables.

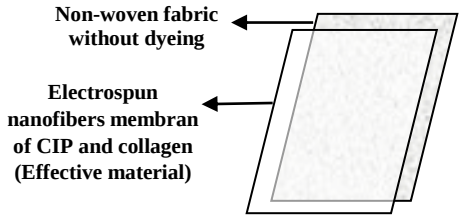


Fig. 1. Design of one-layer sample coated with effective materials. (Represent Sub-groups 1^C&1^D)

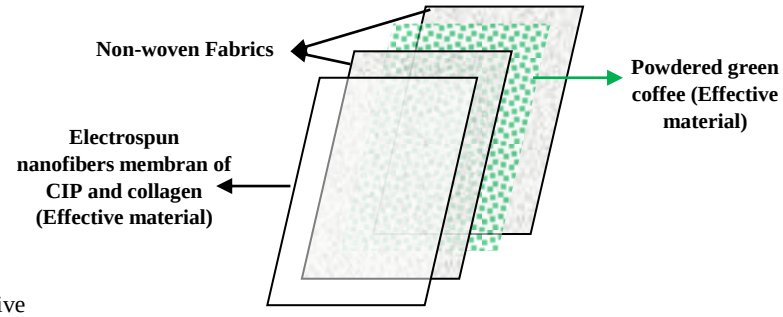
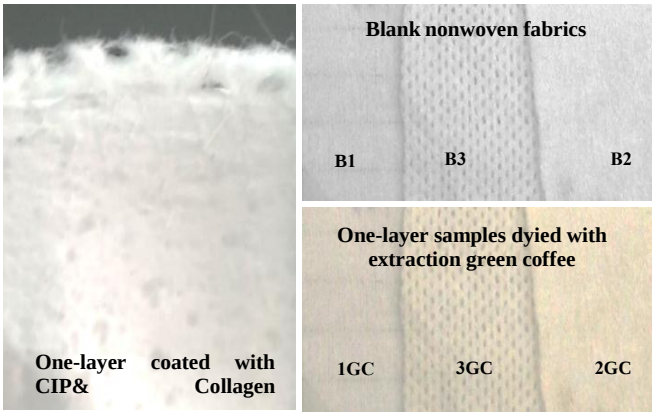


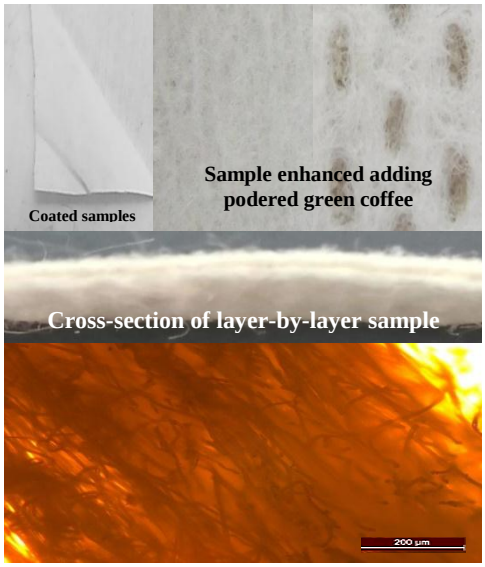
Fig. 2. Design of layer-by-layer sample enhanced with different effective materials. (Represent Sub-groups 2^C&2^D)

2.2.4. Composite Dressing Nonwoven Testing

The physical and mechanical properties of different nonwoven fabrics were tested before and after dyeing, enhancing with green coffee, and electrospun coating membrane. All of the samples were put in the standard atmospheric conditions for 24 hours before testing according to ISO-139. The tests carried out on the fabric samples were as follows: Mass per unit area according to ASTM-D3776-96, Fabric thickness according to ASTM-D1777-96 using Teclock-100125 Digital thickness gauge, Air permeability according to ASTM-D737-96 using Toyoseiki instrument-Japan, Bursting strength of nonwoven samples according to ASTM-D3786 using M229 Autoburst tester- SDL ATLAS, Fabric stiffness by Universal Wear Tester, Toyoseiki -Japan, according to the Gurley method JIS-L 1018, and Absorbency of textiles according to AATCC test method 79-1992.



A: Preparations of one-layer sample



B: Preparations of layer-by-layer sample

Fig. 3: Preparations of samples; A: One-layer sample and B: Layer-by-layer sample

Table 2. Description of final designed samples.

	Sub-group	Code	Description
Main Group 1	Each sample formed from *one layer* of nonwoven fabrics		
	A	B1	**Blank** 100% Cotton.
		B2	Blank 100% Viscose.
		B3	Blank Viscose/Polyester (70:30)%.
	B	1GC	100% Cotton dyed with Green Coffee extraction.
		2GC	100% Viscose dyed with green coffee extraction.
		3GC	100% Viscose/Polyester (70:30)% dyed with green coffee extraction.
	C	$1AC/T^{0.4}$	100% Cotton coated with 0.4g CIP Antibiotic/100mL PVA and 5mL Kylie Collagen.
		$2AC/T^{0.4}$	100% Viscose coated with0.4g CIP Antibiotic/100mL PVA and 5 mL Kylie Collagen.
		$3AC/T^{0.4}$	Viscose/Polyester (70:30)% coated with0.4g CIP Antibiotic/100mL PVA and 5mL Kylie Collagen.
	D	$1AC/T^{0.1}$	100% Cotton coated with 0.1g CIP Antibiotic/ 100 mL PVA and 5 mL Kylie Collagen.
		$2AC/T^{0.1}$	100% Viscose coated with 0.1g CIP Antibiotic / 100mL PVA and 5mL Kylie Collagen.
		$3AC/T^{0.1}$	Viscose/Polyester (70:30)% coated with 0.1g CIP Antibiotic/ 100mL PVA and 5mL Kylie Collagen.
Main Group 2	Each sample formed from *two layers* (layer-by-layer) of nonwoven fabrics		
	A	B12	Blank 100% Cotton layer attached with 100% Viscose layer.
		B13	Blank 100% Cotton layer attached with Viscose/Polyester (70:30)% layer.
		B23	Blank100% Viscose layer attached with Viscose/Polyester (70:30)% layer.
	B	12PGC	100% Cotton layer attached with 100% Viscose layer enhanced adding Green Coffee Powder.
		13PGC	100% Cotton layer attached with Viscose/Polyester (70:30)% layer enhanced adding Green Coffee Powder.
		23PGC	100% Viscose layer attached with Viscose/Polyester (70:30)% layers enhanced adding Green Coffee Powder.
	C	$12AC/T^{0.4}$	100% Cotton layer attached with 100% Viscose layer enhanced adding Green Coffee Powder and coated with 0.4g CIP Antibiotic/100mL PVA and 5mL Kylie Collagen.
		$13AC/T^{0.4}$	100% Cotton layer attached with Viscose/Polyester (70:30)% layer enhanced adding Green Coffee Powder and coated with 0.4g CIP Antibiotic/100mL PVA and 5mL Kylie **C**ollagen.
		$23AC/T^{0.4}$	100% Viscose layer attached with Viscose/Polyester (70:30)% layer enhanced adding Green Coffee Powder and coated with 0.4g CIP Antibiotic/100mL PVA and 5mL Kylie Collagen.
	D	$12AC/T^{0.1}$	100% Cotton layer attached with 100% Viscose layer enhanced adding Green Coffee Powder and coated with 0.1g CIP Antibiotic/ 100mL PVA and 5mL Kylie Collagen.
		$13AC/T^{0.1}$	100% Cotton layer attached with Viscose/Polyester (70:30)% layer enhanced adding Green Coffee Powder and coated with 0.1g CIP Antibiotic/100mL PVA and 5mL Kylie Collagen.
		$23AC/T^{0.1}$	100% Viscose layer attached with Viscose/Polyester (70:30)% layer enhanced adding Green Coffee Powder and coated with 0.1g CIP Antibiotic/ 100mL PVA and 5mL Kylie Collagen.

The color strength values were characterized after dyeing the samples of one-layer to ensure the adsorption of green coffee extraction using the double beam spectrophotometer, according to ASTM E313-96, to obtain the color strength (K/S) measured by using CIE color system coordinates

and the Reflectance (R%), the relation between reflectance and absorbance was given by Kubelka–Munk theory represented by the following equation (1);

$$K/S = (1-R)^2/2R \qquad \text{Eq.(1)}$$

Where; R: Decimal fraction of the reflectance of dyed samples, K: Absorption coefficient, and S: scattering coefficient.

The morphologies of the blank, dyed, enhanced with green coffee, and coated webs were observed using a scanning electron microscope (SEM) using a Philips XL30 scanning electron microscope (SEM) equipped with a LaB6 electron gun and a Philips-EDAX/DX4. Surface morphologies were imaged at different magnifications under the clarity of the images, using 30kV accelerating voltage. Fabric samples were fixed with carbon glue and metalized by gold vapor deposition to record images.

Fourier Transform Infrared Spectroscopy (FT-IR) is used to study the chemical changes and interaction phases in the blank dyed and coated samples. An FTIR spectrum was measured using the ATR technique based on the FTIR spectrometer model (JASCO FT-IR-6100), the spectral range 4000-400 cm^{-1} was recorded.

The tests were carried out in the Spinning and Weaving Engineering Department Laboratories, Scientific Centre of *Excellence* Textile Laboratories, and Central Unit for Analysis and Scientific Services at National Research Centre.

2.2.4.1. Antimicrobial Activity:

Antibacterial activity against *S. aureus* (G+ve) and *E. coli* (G-ve) was evaluated by using the colony counting method [39] where; a liquid culture was prepared by mixing 0.5 g peptone and 0.3 g beef extract in 100 mL water. 1 cm diameter nanofibers were cut and put into 10 mL of liquid culture, to which 10µL of microbe culture was inoculated. All samples were incubated for 24 h at 37°C. From each incubated sample, 100 µL of the solution was taken, diluted, and distributed onto an agar plate. All plates were incubated for 24 h and the colonies formed were counted. All results were expressed after doing a comparison between the control sample and treated samples according to the following equation (2);

$$Reduction\ in\ CFU\ \% = \frac{C-A}{C}\ x100 \qquad \text{Eq.(2)}$$

Where; **CFU:** colony forming units, **C**: the number of microorganisms present on the control sample, and **A:** the number of microorganisms present on the treated samples.

2.2.4.2. Assay for cytotoxicity test of collagen (In-vitro)

2.2.4.2.1. **Cell culture**

The culture was maintained in Dulbecco's Modified Eagle's medium (DMEM) medium (in case of A549), and supplemented with 10% fetal bovine serum at 37°C in 5 % CO_2 and 95% humidity, cells were sub-cultured using trypsin versene 0.15 %. Notable, skin normal human cell line (BJ-1) " Immortalized normal foreskin fibroblast cell line "was obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. Other cell lines "were obtained from Vacsera (Giza, Egypt).

2.2.4.2.2. **Cell viability Assay**

After about 24 h of seeding 20000 cells per well in the case of A-549 cells (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 24 h. 100 µg/ml doxorubicin was used as a positive control and 0.5 % distilled water was used as a negative control. Cell viability was determined using the MTT (3-(4, 5-Dimethylthiazol-2- yl)-2, 5-diphenyltetrazolium bromide) assay as described by Mosmann 1983 with minor modifications as shown in equation (3) [40].

$$Percent\ cytotoxicity = [1 - \frac{Av(x)}{Av(NC)}] \times 100 \qquad \text{Eq.(3)}$$

Where; Av: average, X: absorbance of the sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

2.2.5. Statistical Analysis:

The statical analysis of designed samples was carried out before and after modifications based on their physical and mechanical properties by analysis of variance (ANOVA) by two factors with replicates, the significance level was set at $P \leq 0.05$. In addition, using radar chart evaluation to obtain the best-designed samples for applying the antimicrobial activity and SEM.

3. Results and Discussions

This work is intended to study the behavior of different materials of nonwoven fabrics samples either that formed from one and two layers, modified with Green Coffee, CIP, and Collagen. Also, the results of the designed samples were compared together in terms of mechanical, physical, morphological, and biological properties, due to determine the performance of the best-designed sample to be used as the inner layer of endoscopic dressing. The results of nonwoven samples' properties are shown as follows:

3.1. Weight Test Results:

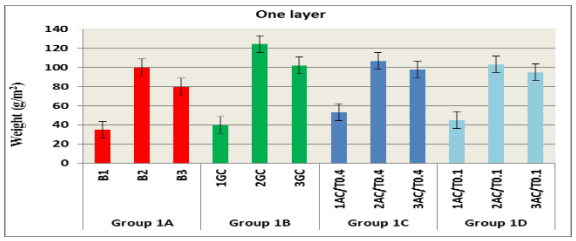


Fig. 4A. Weight test results of group one samples.

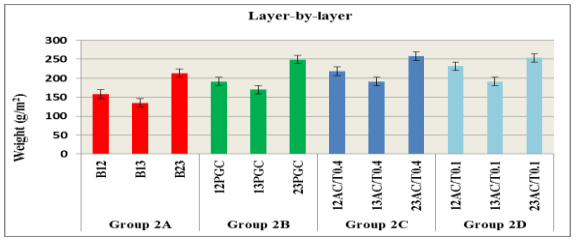


Fig. 4B. Weight test results of group two samples.

Table 3. Weight gain (%) compared between the blank samples results and the two groups samples after different modifications

One-layer samples (Fig. 4A)		Weight gain (%)	Layer-by-layer samples (Fig. 4B)		Weight gain (%)
Group 1B	1GC	14.29	Group 2B	12PGC	20.96
	2GC	24.4		13PGC	25.51
	3GC	27.75		23PGC	16.85
Group 1C	1AC/T $^{0.4}$	52.37	Group 2C	12AC/T $^{0.4}$	37.83
	2AC/T $^{0.4}$	6.66		13AC/T $^{0.4}$	41.56
	3AC/T $^{0.4}$	22.21		23AC/T $^{0.4}$	21.02
Group 1D	1AC/T $^{0.1}$	28.57	Group 2D	12AC/T $^{0.1}$	46.27
	2AC/T $^{0.1}$	3.22		13AC/T $^{0.1}$	41.56
	3AC/T $^{0.1}$	18.63		23AC/T $^{0.1}$	18.94

Fig. 4A shows the increase in weight value for all treated samples of group 1^B was from 14.29-27.75 % see Table 3, which referred to the dyed samples with green coffee extraction, due to the swelling of the fibers during the dyeing process, by the interaction between the component of green coffee extraction within fibers and their fixing by the Alum. After that came, the samples of groups 1^C and 1^D were coating with CIP and Kylie Collagen nanofibers membrane according to the weight values. Also, it was noted that the cotton samples with coating modifications ($1AC/T^{0.4}$, and $1AC/T^{0.1}$) had the highest weight value ranged from 52.37- 28.57 % see Table 3, followed by viscose/polyester (70:30) % samples in all groups 22.21-18-63 % in Table 3.

Fig. 4B presents an increase of weight (%) for the sample groups that formed from layer-by-layer after modifications compared to group 1^A of blank samples that was from 46.27- 16.85% see Table 3. This could be related to the addition of green coffee powder between the layers in group 1^B and coating with Collagen and CIP nanofibers membrane for groups 1^C & 1^D. In addition, the samples (13) made of cotton/viscose blended polyester layers in most groups showed the highest weight values, followed by the sample (12) consists of cotton/viscose layers compared to other materials of samples, due to the ability of cellulosic materials to absorbency and the mass per unit area for each sub-layer material of sample, as referred in Table 1.

In general; the difference between designed samples in results of weight depends on the weight of blank samples as an initial reason as referred to in Table 1, and the density of the material used, where the increase in the density of materials reflects caused increase in weight value [41]. In addition to, the ratio of dyeing bath for one-layer samples, the amount of enhanced material for layer-by-layer design, and finally the ratio of the electrospun coating materials.

3.2. Thickness Test Results

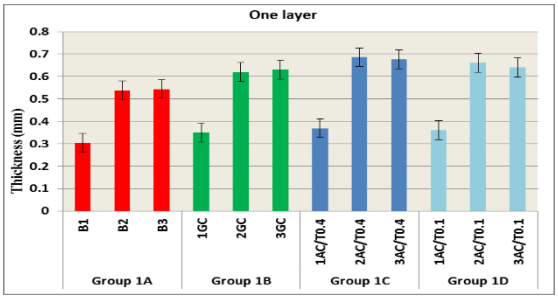


Fig. 5A: Thickness test results of group one samples.

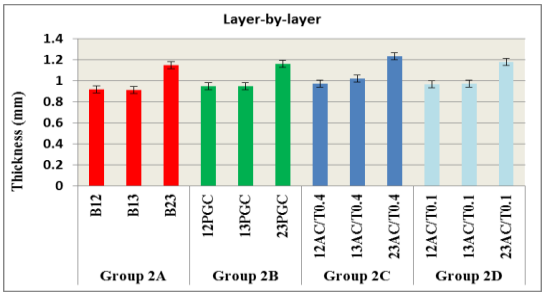


Fig. 5B: Thickness test results of group two samples.

Table 4. Thickness gain (%) compared between the blank samples results and the two groups samples after different modifications

One-layer samples (Fig. 5A)		Thickness gain (%)	Layer-by-layer samples (Fig. 5B)		Thickness gain (%)
Group 1B	1GC	15.13	Group 2B	12PGC	3.26
	2GC	15.03		13PGC	4.39
	3GC	15.81		23PGC	0.87
Group 1C	1AC/T $^{0.4}$	21.38	Group 2C	12AC/T $^{0.4}$	5.79
	2AC/T $^{0.4}$	27.27		13AC/T $^{0.4}$	12.09
	3AC/T $^{0.4}$	24.45		23AC/T $^{0.4}$	7.25
Group 1D	1AC/T $^{0.1}$	18.75	Group 2D	12AC/T $^{0.1}$	4.71
	2AC/T $^{0.1}$	22.82		13AC/T $^{0.1}$	6.59
	3AC/T $^{0.1}$	17.83		23AC/T $^{0.1}$	2.61

It was observed that the samples of group 1^{c} coated by electrospun nanofibers membrane gave the highest values of thickness, especially for the samples 2AC/T0.4 (100% viscose coated with 0.4 g CIP antibiotic and collagen) that achieved (27.27)% increase of thickness, followed by $3AC/T^{0.4}$ (viscose/polyester coated with 0.4g CIP and collagen) about (24.45)% related to the blank sample, as shown in Fig. 5A and Table 4. Followed by the samples of group 1^{D}, especially the sample 2AC/T (100% Viscose coated with 0.1g CIP and collagen) that achieved about (22.82)% of thickness gain. This is related to the antibiotic ratio and collagen of electrospinning nanofibers coating. Also, it was noted that the samples from (100% viscose) with coating membrane recorded the highest thickness values, due to the mass per unit area of viscose material as referred to in Table 1, in addition to the effect of different modification processes.

The thickness results of dyed samples with green coffee extraction increased with closed values compared to the blank samples, This is related to the swelling that occurred for fibers in the nonwoven fabric as a result of dyeing and the ratio of nanofibers coating membrane.

While the Fig. 5B and Table 4 show that the greatest thickness values for the sample $13AC/T^{0.4}$ (cotton/ viscose blended with polyester layers and enhanced with powdered green coffee, then coated with 0.4g CIP and collagen) in group 2^{c} compared to the blank samples of group 1^{A} and other modified samples of groups 2^{B} &2^{D}, due to the physical crosslinking within electrospun nanofibers coating that formed a web with 0.4g ratio of antibiotic. Also, the samples of group 2^{B} that were enhanced with powdered green coffee only gave the lowest thickness gain percent compared to the blank samples of group 2^{A}, where the increase in thickness was related to the density of the nonwoven fabric and the amount of green coffee powder.

Generally, the thickness gain percentage of samples in Fig. 5A was higher than in Fig. 5B, whereas the sample from one layer was affected with different modifications with the density of nonwoven fabrics as mentioned in Table 1. So, the difference in values was clear. While the layer-by-layer samples were affected with multiparameter such as; materials design, the amount of powdered green coffee between layers, and total densities of layers.

3.3. Air Permeability Test Results:

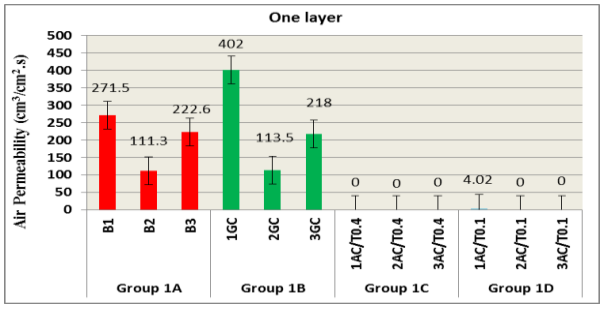


Fig. 6A. Air permeability test of group one samples.

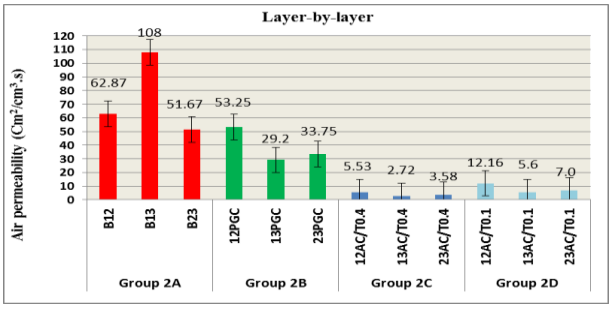


Fig. 6B. Air permeability test results of group two samples

Fig. 6A shows that the air permeability rates were increased for the dyed samples with green coffee extraction of group 1^{B}, especially for the 1GC sample (100% cotton). This may be due to the scouring process that removes any contamination's in nonwoven fibres before dyeing which leads to widening of the internal gaps between the chains that build the fibers, which allows air to pass at a

high rate [42]. The samples of groups 1^{C}&1^{D} didn't record air permeability value, due to the coating layer of CIP antibiotic and collagen, which blocked the pores of the surface of the fabric.

While Fig. 6B showed a decrease in air permeability rates compared to the samples in Fig. 6A. This is related to the number of layers of the designed sample and the enhancement with powdered green coffee. Also, the samples of group 2^{B} that were enhanced with powdered green coffee only were decreased compared to the blank samples of group 2^{A} because of the obstruction caused by the powdered green coffee between the layers of the sample. The samples of groups 2^{C} &2^{D} achieved low values of air permeability smaller than 15 cm^3/cm^2.s respectively, compared to the samples of groups 2^{A} &2^{B}, due to the coating with CIP and collagen.

Also, it was noted that the groups' samples 2^{C}& 2^{D} (Fig. 6B) gave lower air permeability rates compared to the groups' samples 1^{C}&1^{D} (Fig. 6A), as didn't record air permeability values. This is due to the addition of powdered green coffee that trapped between the layers of each sample, causing an increase in the sample thickness and allowed air exchanges by air tracks between the layers of the sample

3.4. Bursting Strength Test Results:

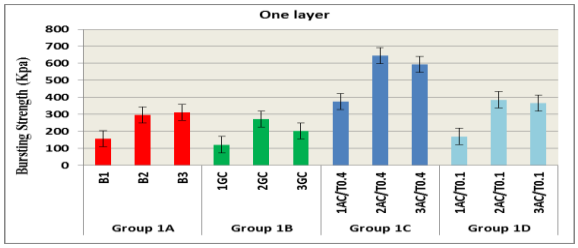


Fig. 7A: Bursting strength of group one samples.

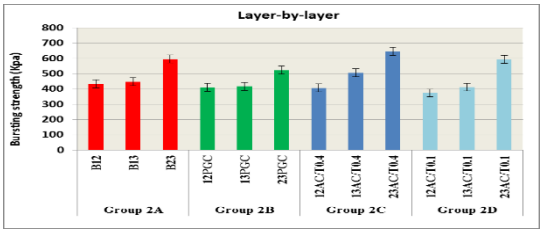


Fig. 7B: Bursting strength of group two samples.

Table 5. Bursting strength changes (%) compared between the blank samples results and the two groups after different modifications

One-layer samples (Fig. 7A)		Bursting strength changes (%)	**Layer-by-layer samples (Fig. 7B)**		Bursting strength changes (%)
Group 1B	1GC	-21.92	Group 2B	12PGC	-5.69
	2GC	-7.68		13PGC	-6.64
	3GC	-35.01		23PGC	-12.22
Group 1C	1AC/T $^{0.4}$	140.81	Group 2C	12AC/T $^{0.4}$	-6.59
	2AC/T $^{0.4}$	118.43		13AC/T $^{0.4}$	13.26
	3AC/T $^{0.4}$	90.75		23AC/T $^{0.4}$	8.22
Group 1D	1AC/T $^{0.1}$	9.25	Group 2D	12AC/T $^{0.1}$	-13.65
	2AC/T $^{0.1}$	30.40		13AC/T $^{0.1}$	-7.86
	3AC/T $^{0.1}$	17.67		23AC/T $^{0.1}$	-0.60

Fig. 7 (A&B) and Table 5 point to the values of bursting strength that was close, especially for the final samples designed from two layers, this is due to the layer-by-layer design used, and the enhancement adding powdered green coffee between the layers of samples that affected on the strength resistance samples.

In Fig. 7A the samples of group 1^{B} (dyed with green coffee extraction) gave the lowest values of bursting strength compared to group 1^{A} of blank samples and the modified samples of groups' 1^{C}&1^{D}, caused by the dyeing with green coffee extraction that affected on the mechanical properties as; strength and stiffness…*etc.* Thus, the scouring by NaOH is primarily responsible for the weak mechanical properties of the fibers before dyeing, which causes the weak points along with the fiber. Additionally, the drying process is carried out by autoclave [42, 43].

While the samples of group 1^{C} gave the highest values of bursting strength, due to the coating nanofibers membrane, which contains CIP with 0.4g ratio and collagen that supported the strength of samples because of the crosslinking of electrospun nanofiber coating layer through functional groups of CIP and collagen. Also, it was noted that sample 1 (100 % cotton) with different modifications showed the highest increase % of bursting strength than the other samples with the same modification compared to the blank samples.

Fig. 7B shows that the 13 $AC/T^{0.4}$ sample of group 2^{C} gave the highest value of bursting strength, followed by the 23AC/T0.4 sample of group 2^{C}, due to the properties of the viscose as a regenerated material with the crystalline region for the sample 13 and the ratio of polyester fibers in sample 23 that characterized by its high strength [44].

Generally, the modified samples showed the lowest burst strength values compared to the blank samples group for the dyed samples in Fig.s 7A and samples that enhanced adding green coffee powder only and those coated with 0.1g CIP and collagen nanofibers membrane in Fig. 7B, caused by the

design of the final sample that has more air gaps and crossed air tracks between the sub-layers of the sample.

3.5. Stiffness Test Results:

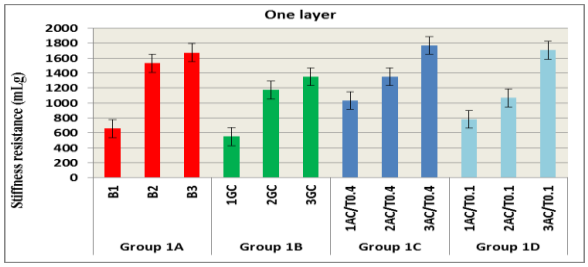


Fig. 8A. Stiffness values of group one samples.

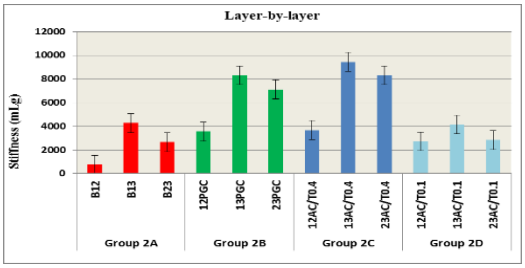


Fig. 8B. Stiffness values of group two samples.

Table 6. Stiffness changes (%) compared between the blank samples results and the two groups after different modifications

One-layer samples (Fig. 8A)		Stiffness changes (%)	Layer-by-layer samples(Fig. 8B)		Stiffness changes (%)
Group 1B	1GC	-16.22	Group 2B	12PGC	376.19
	2GC	-23.26		13PGC	94.44
	3GC	-19.14		23PGC	166.67
Group 1C	1AC/T $^{0.4}$	56.76	Group 2C	12AC/T $^{0.4}$	392.06
	2AC/T $^{0.4}$	-11.63		13AC/T $^{0.4}$	120.83
	3AC/T $^{0.4}$	5.79		23AC/T $^{0.4}$	211.11
Group 1D	1AC/T $^{0.1}$	18.92	Group 2D	12AC/T $^{0.1}$	265.08
	2AC/T $^{0.1}$	-30.23		13AC/T $^{0.1}$	-2.78
	3AC/T $^{0.1}$	1.91		23AC/T $^{0.1}$	6.67

Fig. 8A and Table 6 point to the stiffness values for the one-layer samples, the group's samples 1^{B} that dyed with green coffee extraction showed the lowest values of stiffness, maybe due to the dyeing with green coffee extraction, which affected the links between cellulose chains by weakling during scouring and dyeing process. The fabrics' stiffness decreased with the increase of NaOH concentration [42]. The sample of groups 1^{D}&1^{C} that coated with two ratios of CIP and collagen, respectively achieved high resistance of stiffness, especially for the samples $1AC/T^{0.4}$ and $1AC/T^{0.1}$ (based on cotton), due to the strength and support of the electrospun nanofibers coating membrane, additionally to the tenacity of cotton increased with wet process. While the samples $2AC/T^{0.4}$ &$2AC/T^{0.1}$ (based on viscose) had negative changes in stiffness, due to the nature of viscose as a regenerated material that is characterized by smoothness and handling.

Fig. 8B and Table 6 present high stiffness for all samples compared to the samples groups in Fig. 8A, this could be related to the layer-by-layer design with different parameters used. In addition to, the enhancement of the layers with powdered green coffee. The samples of group 2^{C} gave the highest stiffness values; this is due to the different ratios of CIP that were added with collagen in the nanofibers coating membrane. We can note also that, sample 13 (cotton/ viscose layers) in groups 2^{C}, 2^{B}, and 2^{D}, respectively achieved the highest resistance values of stiffness compared to the other samples materials, whereas it contains two layers of nonwoven that have high densities for cellulosic material and powdered green coffee materials, which increased the thickness of samples that affected on stiffness values.

3.6. Absorbency Test Results

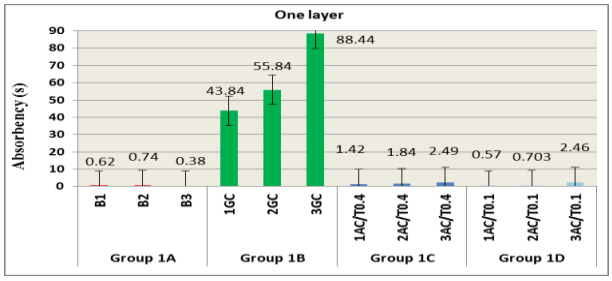


Fig. 9A. Absorbency time of group one samples.

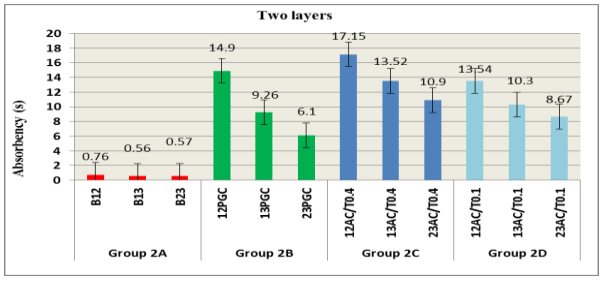


Fig. 9B. Absorbency time of group two samples.

Fig. 9A shows that the samples of group 1^B that dyed with green coffee extraction gave the longest time for absorbency; this is due to the dyeing process, which caused swilling of fibers within nonwoven fabrics resulting in the saturation that occurred during the dyeing bath. Also, the absorption for the samples of the group 1^B takes place at the site of the droplet and did not spread rapidly due to the proportion of oil that is found in the coffee extract used for dyeing, which increased the absorbency time of the droplet.

While Fig. 9B shows that the samples' of groups 2^D & 2^C that coated with two different ratios of CIP added with collagen and enhanced with powdered green coffee, respectively gave the longest absorbency time compared to the group 2^B samples that enhanced only with powdered green coffee. Sample 12 (cotton/ viscose layers) in all sub-groups achieved the longest absorbency time compared to other samples because cotton material has high absorption capacity, so it takes a long time for impregnation and saturation of water. Additionally, the dyeing process caused the saturation for nonwoven fibers, which also increase the time of absorbency. While viscose and viscose/polyester materials have a faster, ability to penetration not absorption caused by a high-crystallize region.

3.7. The effects of samples design on the measured properties;

3.7.1. One-layer samples

Table 7. Significance of one-layer samples properties

Variation		*Weight*	*Thickness*	*Air Permeability*	*Bursting Strength*	*Stiffness*	*Absorbency*
Different materials samples in each group.	**F**	2983.24	38.12	3429.84	75. 3	56.15	5.12
	P-value	1.71E-29	1.23E-38	3.23E-30	4.55E-11	8.88378E-10	0.014096
	Significance	**	**	**	**	**	*
Between the modified samples in groups.	**F**	40.15	887.68	13428.39	103.46	5.17	96.72
	P-value	1.64E-09	9.44E-13	8E-39	7.25E-14	0.00675156	1.54E-13
	Significance	**	**	**	**	**	**
Interaction.	**F**	58.21	8.12	1362.76	4.08	1.77	4.37
	P-value	4.02E-13	4.44E-06	3.57E-29	0.005936	0.147631786	0.004047
	Significance	**	**	**	**	NS	**
*** Significant**			**** Highly-Significant**			**NS: Non-Significant**	

Table 7 points to the significant results for all samples of main group 1 that formed from one layer related to nonwoven fabric and modifications parameters used in work. It was highly significant results for the samples with different materials in each group in terms of weight, thickness, air permeability, bursting strength, and stiffness resistance properties, but significant of absorbency property. Also, the different modifications that were used on the different samples showed a high significance for all measured properties. While it was revealed that, the interaction between nonwoven fabric materials and different modifications applied showed a high significance for all measured properties except stiffness properties.

3.7.1 Layer-by-layer samples

Table 8. Significance of layer-by-layer samples properties

Variation		*Weight*	*Thickness*	*Air Permeability*	*Bursting Strength*	*Stiffness*	*Absorbency*
Different materials samples in each group.	**F**	1181.47	186.84	403.91	183.4	17.02	18.02
	P-value	1.07E-24	2.33E-15	3.33E-19	2.88E-15	2.49E-05	1.67E-05
	Significance	**	**	**	**	**	**
Between the modified samples in groups.	**F**	170.95	11.45	7655.9	14.72	16.80	68.32
	P-value	2.51E-16	7.43E-05	6.74E-36	1.2E-05	4.29E-06	6.74E-12
	Significance	**	**	**	**	**	**
Interaction.	**F**	17.78	10.22	723.1	4.86	1.36	2.33
	P-value	9.24E-08	1.22E-05	6.92E-26	0.00223	0.27057	0.06473
	Significance	**	**	**	**	NS	NS
*** Significant**			**** Highly-Significant**			**NS: Non-Significant**	

Table 8 shows that the significance of test results for all samples of main group 2 that designed from layer-by-layer, the measured properties were showed a highly significant affected with the different materials of nonwoven samples in each sub-group. Also, the modifications were revealed to a highly significant for measured properties. Finally, the interaction between the

different nonwoven samples materials and modifications for samples showed a high significance for measured properties except stiffness and absorbency properties that showed non-significant.

3.8. Radar Evaluation Charts of Designed Dressing Nonwoven:

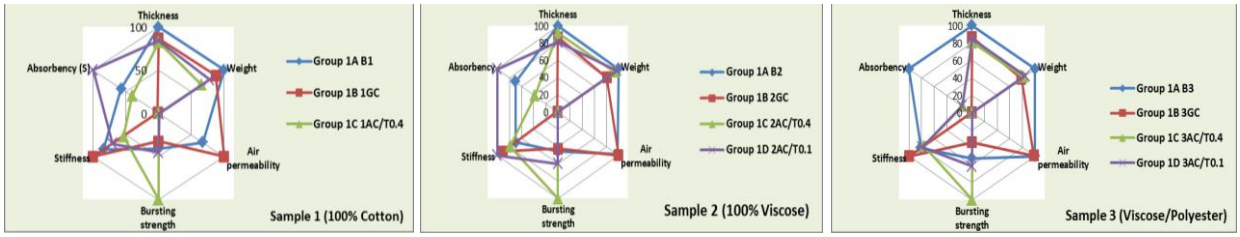


Fig. 10A. Radar evaluation charts for group one of dressing nonwoven.

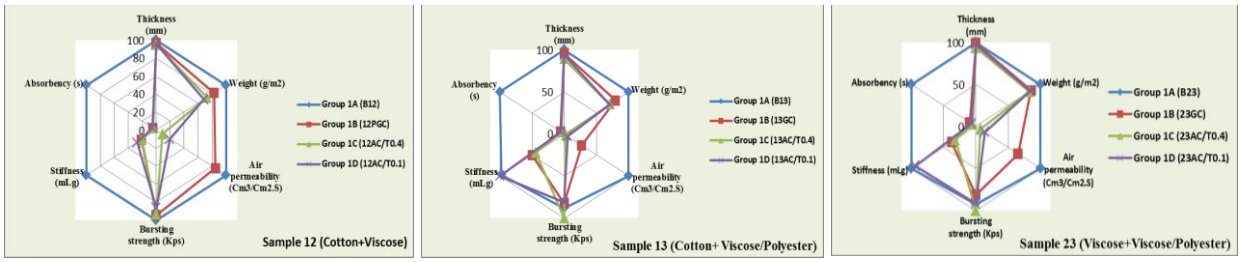


Fig. 10B. Radar evaluation charts for group two of designed dressing nonwoven.

Fig. 10A shows the radar evaluation charts for one-layer samples. The dyed samples with green coffee extraction had the highest area of the radar chart. While Fig. 10B points to the radar evaluation of the designed samples layer-by-layer. It was shown that samples 12 & 23 (cotton/viscose layers & viscose/viscose blended polyester layers enhanced adding green coffee powder) revealed the highest area of radar chart respectively, followed by sample 13 that designed from cotton/viscose blended polyester layers and enhanced adding green coffee powder then coated with 0.1g CIP added with collagen.

In general, we can note the blank samples with different materials either that designed from one-layer or layer-by-layer gave a higher area of radar chart more than the samples after different modifications. This is due to the effect of different modifications on the properties of the sample especially for air permeability with electrospun nanofibers coating membrane, and bursting strength of dyeing samples. In addition, the structure of samples with different modifications gave diverse responses to different measured properties especially with the samples designed layer-by-layer.

3.9. Fourier Transform Infrared Spectroscopy (FTIR)

Fig. 10 A & B shows FT-IR spectra of a different design of dressing samples; which either consists of one-layer or layer-by-layer with various enhanced materials. Fig. 11A represents the FT-IR chart of one-layer of cotton, viscose, and viscose/polyester (70:30)% blended fabrics as B1, B1, and B3 respectively and their treated samples with green coffee extraction, 5 mL Kylie collagen and CIP antibiotic with two different concentrations at 0.1 and 0.4 wt%. Whereas, Fig. 11B represented the FT-IR of layer-by-layer of cotton-viscose, cotton-viscose/polyester blended fabrics, and viscose-viscose/polyester blended fabrics as matrices were loaded with green coffee, Kylie collagen, and CIP antibiotic (detailed data of each sample represent in Table 2). Fig. 11A shows similar bands at 3273 cm^{-1} corresponding to OH stretching vibration band, while the band at 2932 cm^{-1} corresponds to C – H stretching vibration band and the two bands at 1700 cm^{-1} corresponding to the non-hydrated carbonyl group (- C = O). In addition, the band at 1350 cm^{-1} is due to C – OH – a bond of pyranose ring for B1, B2, and B3 for cellulosic-based fabrics (cotton, viscose, and viscose-polyester blende fabrics). In addition, the band peaks of treated fabrics with green coffee showed similar band peaks at the OH stretching region and the disappearance or dimensionless at some peaks at CH stretching and C=O stretching due to functional groups cross interaction [45]. The FT-IF spectra of ciprofloxacin showed main peaks at 3490, 3320, 2930, 2840, 1696, 1605, 1480, and 1435 cm^{-1} that results from the similarity of FT-IR spectra charts for both B1, B2, and B3 treated fabrics with green coffee and ciprofloxacin that confirm the presence of green coffee and ciprofloxacin on cellulose-based fabrics [46-48]. Also Fig. 11B shows typical band peaks for double layers of cotton/viscose (12), cotton/viscose-polyester (13), and viscose/ viscose-polyester (23) fabrics and their treated fabrics with green coffee and two different concentrations of ciprofloxacin antibiotics [47].

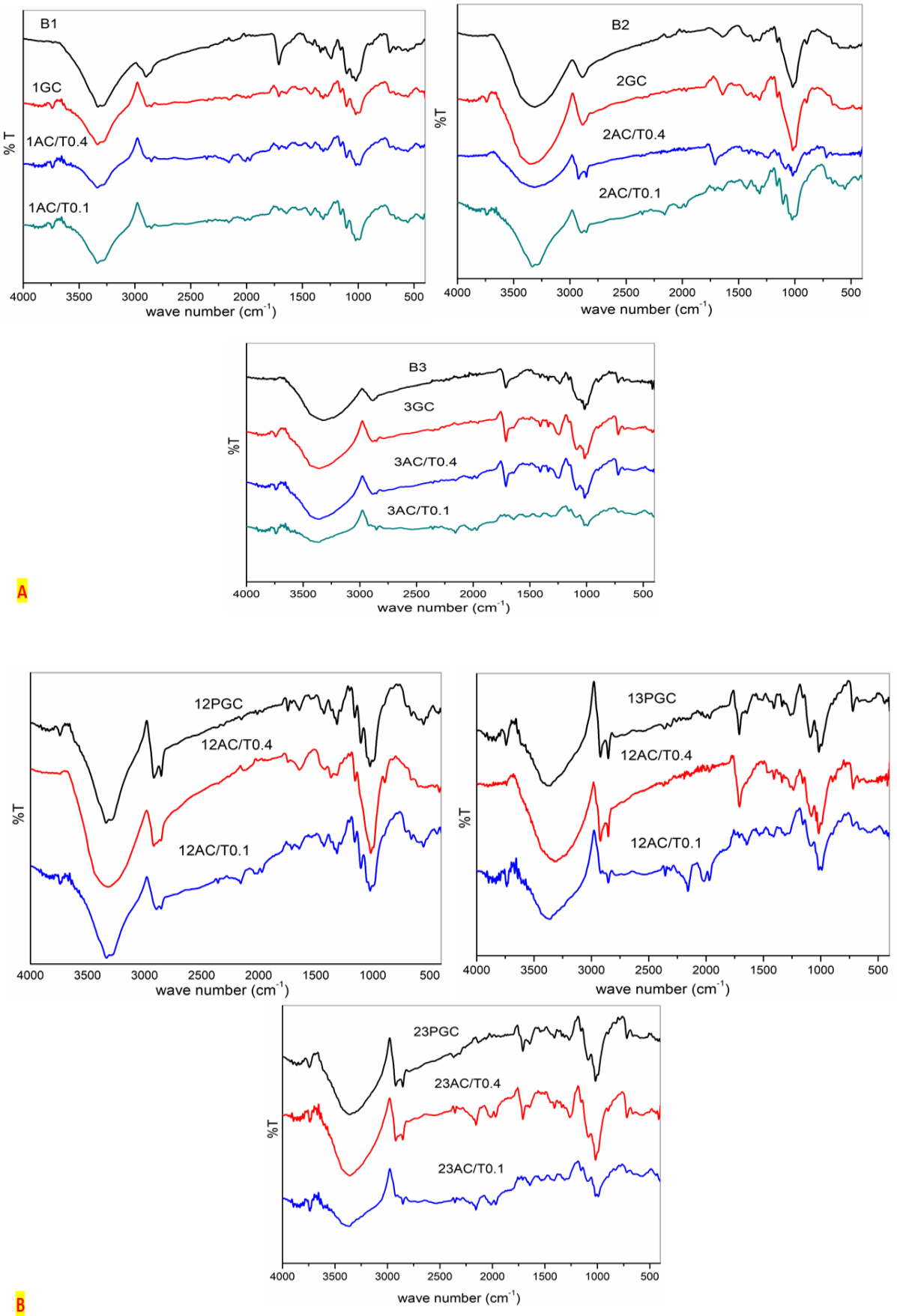


Fig. 11: FT-IR charts of one-layer fabrics (A) and layer-by-layer fabrics (B) and their modified samples with green coffee, Kylie Collagen, and CIP antibiotic

3.10. Color Characteristics:

Table.: K/S values of one-layer samples dyed with green coffee extract

Dyed Samples (one layer)	K/S	R%
B1	0.39	1.463
B2	1.55	3.394
B3	1.49	3.284
1GC	1.34	3.012
2GC	2.89	5.948
3GC	2.46	5.115
Wavelength	**350 nm**	

Table 9 shows the wavelength of maximum absorption of the green coffee dye was at 350 nm. The majority of natural dyes need a mordant in the form of a metal salt to create an affinity between the fiber and the pigment, these metals form a ternary complex on one side with the fiber and on the other side with the dye. Such a strong coordination tendency enhances the interaction between the fiber and the dye that is measured by color Strength, which is known as *K*/*S* dyed.

It was noted that the optimum condition for the highest color response was K/S (2.89) with viscose material. These results may be due to the maturity % and water absorbency of the viscose fabric as a modified cellulosic material, which indicates that there is more precipitated cellulose (crystalline or amorphous), thus producing more free hydroxyl groups (OH) which can react with the extracted dye to give high K/S value. While that the lowest color response K/S (1.34) was found with cotton material, this is related to mainly the density and weight of the available cotton nonwoven fabric. The increase in color properties can be explained generally because of the destruction of crystalline regions during swelling that leads to changes in microstructure and morphology of nonwoven samples [49, 50].

3.11. Scanning Electron Microscopy (SEM):

Fig. 12**A** shows the SEM of best samples treated with green coffee compared with blank samples cotton (A), viscose (B), and polyester/viscose (C). The cotton samples modified with green coffee in both one-layer samples dyed samples with green coffee extraction and two layers samples enhanced with powdered green coffee between the layers, represented in Fig. 12 (D) and (E), respectively are coated inhomogeneous matter that led to homogeneous treatment that will be reflected in higher antibacterial activity and lower cytotoxicity.

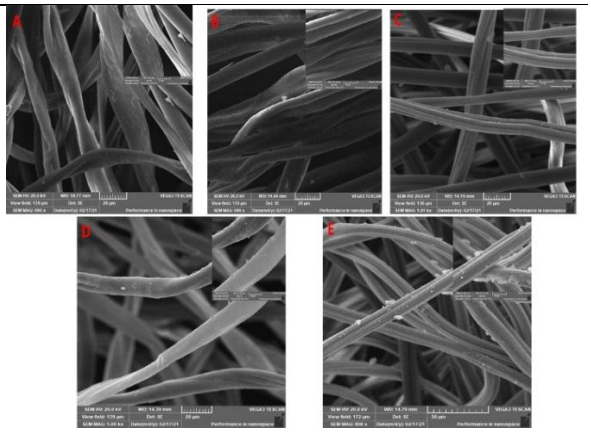


Fig. 12A. SEM of untreated fabrics; cotton (A), viscose (B), polyester/viscose (C), dyed one layer with green coffee extraction of the cotton sample (D) and layer-by-layer of cotton enhanced adding green coffee powder (E)

Fig. 12B SEM of electrospun nanofibers samples of one-layer of viscose/polyester blended samples dyed with 1 v/v% green coffee and 0.4 wt% ciprofloxacin (A) and layer-by-layer of viscose and viscose/polyester blended fabric treated with 1 v/v% green coffee and 0.1 wt% ciprofloxacin (B). Fig. 12B shows that both green coffee between layers and CIP antibiotic are regularly distributed within electrospun coating nanofibers where the diameter ranges from 280-400nm that improved the highest values of physicochemical and mechanical properties of the prepared dressing samples. Regular distribution of green coffee particles from and CIP antibiotics make it gives a very high surface area and antibacterial activity.

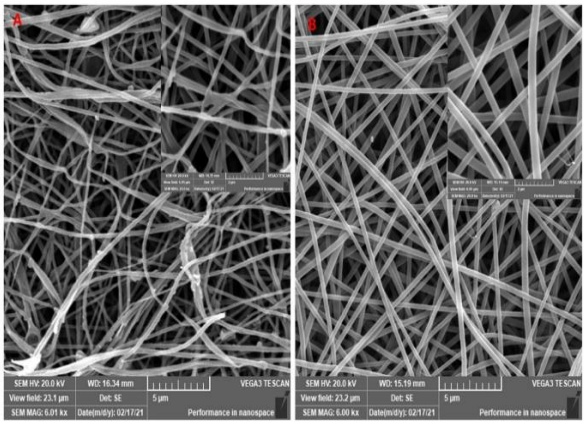


Fig. 12B. SEM of electrospun nanofibers samples of one-layer of viscose/polyester blended samples dyed with 1 v/v% green coffee and 0.4 wt% CIP (A) and layer-by-layer samples of viscose and viscose/polyester blended fabric dyed with 1 v/v% green coffee and treated with 0.1 wt% CIP (B)

3.12. Antibacterial Activity Results

Table 10 illustrates the results of antibacterial activity; the first group of dyed samples that consisting of one layer of nonwoven fabrics showed high antibacterial activity, this is due to the effect of green coffee extraction on bacterial resistance as it has antibacterial and antioxidants properties and Alum has an astringent effect and therefore is used to stop bleeding [27-29, 51, 52], especially the sample 3GC (based on viscose/polyester) against the Gram-positive bacteria (*S. aureus*) and the gram-negative bacteria (E. coli), which due to the effect of antibacterial activity of polyester material.

It is also evident from Table 10 that the layer-by-layer samples achieved good results for antibacterial activity, but the results of dyeing samples with green coffee extraction were still higher, while it was found that the layer-by-layer samples that coated by electrospinning with CIP antibiotics and Kylie collagen had increasing antibacterial activity, especially with the ratio of CIP antibiotics 0.4 as in sample 13AC/T0.4 (cotton/ viscose blended polyester).

In general, dyeing with green coffee extraction increased the reduction of bacterial activity, but the samples designed layer-by-layer and coated with nanofibers membrane of CIP and Collagen were significantly superior in results due to the design of samples and the components used to support the inhibition of bacterial activity.

Table 10. Bacterial reduction % of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli)* after 24 h incubation hours

Sample	Code	Bacterial reduction (%)	
		S. aureus	***E. coli***
One-layer	1B	00	00
	2B	00	00
	3B	00	00
	1GC	66.7	55.2
	2GC	70.1	60.4
	3GC	79.2	63.8
Layer-by-layer	12PGC	58.9	57.6
	23PGC	60.5	60.0
	12AC/T $^{0.1}$	86.99	85.90
	23AC/T $^{0.1}$	85.88	83.02
	13AC/T $^{0.4}$	89.88	88.02
Green Coffee Powder		**92.99**	**90.78**
Ciprofloxacin antibiotic (CIP)		**99.99**	**96.78**

3.13. Cytotoxicity of collagen via MMT assay

The cytotoxicity of collagen, green coffee, and ciprofloxacin have been studied carefully before being used as a biomaterial. A549 cells were used for the treatment of different concentrations (0-0.5 wt. %) of collagen, green coffee, and ciprofloxacin expressed in viable cells after 3 and 24 hrs. Cytotoxicity of collagen, green coffee, and ciprofloxacin was evaluated by using the MMT protocol where it was used to evaluate viable A549 cells expressed in mitochondrial activity decrement as shown in Fig. 13. The number of viable cells of collagen and green coffee confirms that these biomaterials are safe and non-cytotoxic compared with these cells for ciprofloxacin antibiotic as shown in Fig. 13.

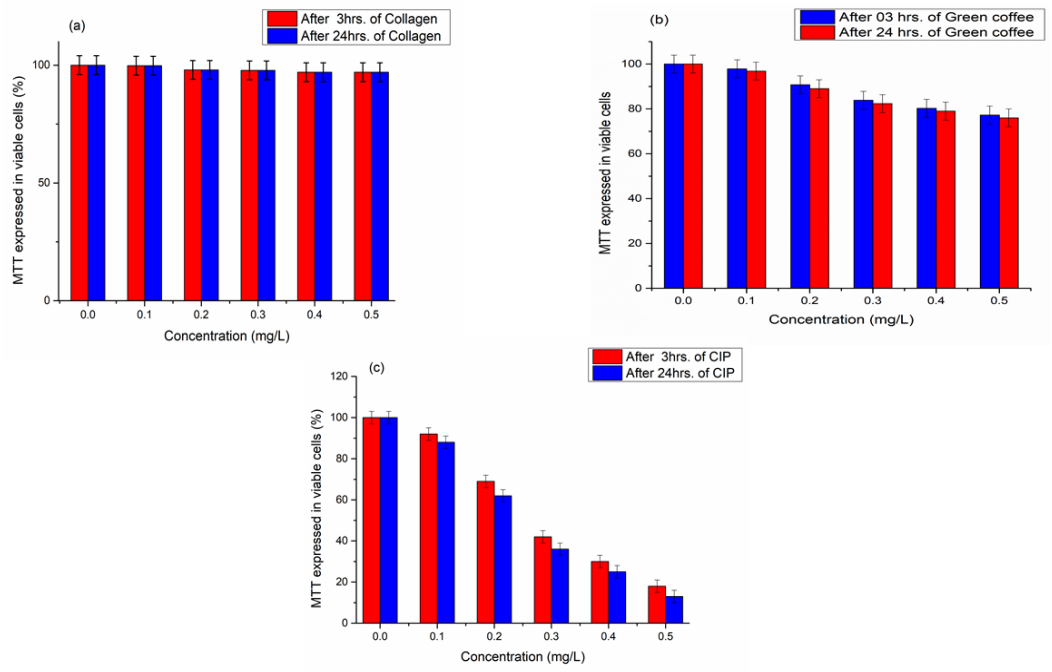


Fig. 13: MMT test (mitochondrial metabolic activity) of collagen (a), green coffee (b), and ciprofloxacin (c) expressed in viable cells after 3 hrs., and 24hrs

Fig. 13 illustrated that there is no significant decrease in viable cells within a range of both collagen and green coffee concentration from 0 to 0.5 wt.%. So, it can be reported that collagen and green coffee are non-toxic, safe, and cell compatible and can be used as a biomaterial for drug delivery.

4. Conclusion

The inner layer dressing samples of research were designed for speeding up and controlling biological fluids leakage from deep endoscopic wounds without any infection as possible using completely safe material transactions. Green coffee was used to dye the one-layer samples and enhancement the layer-by-layer samples of nonwoven fabrics as a disposable use to impart coveted bio-functional properties. Also, Ciprofloxacin Antibiotic and Kylie Collagen substance as nanofibers coating membrane by electrospinning technique help to stimulate cells and reduce the infection.

- In the one-layer samples; different nonwoven materials, various modifications, and interaction between them had a highly significant effect on all measured properties of samples, except the interaction between different materials of samples and modifications types didn't have a significant effect on the stiffness.
- In the layer-by-layer samples; different nonwoven materials, various modifications, and interaction between them had a highly significant effect on all measured properties of samples, except the interaction between different materials of samples and modifications types had a non-significant effect on the stiffness.
- The dyed samples with green coffee extraction achieved a high radar chart area compared to the other samples, while the samples of cotton/ viscose layers and viscose/ viscose blended polyester layers those enhanced with powdered green coffee achieved the highest radar chart area, respectively, followed by the designed sample from cotton/ viscose blended polyester layers that enhanced with powdered green coffee, and coated with 0.1g CIP added with collagen.
- In the antimicrobial activity results; the dyed one-layer samples have a good antimicrobial activity, while the layer-by-layer samples enhanced with powdered green coffee and coated with nanofibers membrane of CIP and Collagen shows high activity against microbial organisms.
- MTT assay showed that collagen and green coffee are non-toxic and tissue compatible materials and have strong antibacterial properties and lower cytotoxicity at the same time, which confirms that they have the potential to be used in biomedical applications.

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