

Electrospun Poly(lactide-co-glycolide)/Nanotube Composite Nanofibers for Drug Encapsulation and Sustained Release

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Abstract: In this report, we present a novel electrospun composite nanofiber-based drug delivery system. In this system, nanotubular materials halloysite nanotubes (HNTs) were first used to encapsulate a model drug, tetracycline hydrochloride (TCH). Then, the drug-loaded nanotubes with an optimized drug encapsulation percentage were mixed with poly (lactide-co-glycolide) (PLGA) polymer solution for subsequent electrospinning to form drug-loaded composite nanofibrous mats. The structure, morphology, and mechanical properties of the formed electrospun composite nanofibrous mats were characterized using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and tensile testing. In vitro drug release behavior was examined using UV-vis spectroscopy. We show that the incorporation of HNTs within the nanofibrous mats does not significantly change the morphology of the mats. In addition, our results indicate that this double-container drug delivery system (both PLGA polymer and HNTs are drug carriers) is beneficial to avoid the burst release of the drug and the introduction of HNTs can improve the tensile strength of the polymer nanofibrous mats. The drug loaded electrospinning composite nanofibrous mats developed in this study may find various applications in tissue engineering and pharmaceutical sciences.

Keywords: poly(lactide-co-glycolide); halloysite nanotubes; tetracycline hydrochloride; composite nanofibers; drug delivery system.

1. Introduction

In recent years, a tremendous progress in the research of tissue engineering and drug delivery has been witnessed due to their unlimited potential to improve human health. Meanwhile, the development of electrospinning nanotechnology provides opportunities for fabricating fibers with a diameter from nanometer to a few micrometers [1-3]. This special dimension endows electrospun fibers with a large specific surface area and a porous structure. Therefore, the electrospun scaffold has the priority to closely mimic the natural extracellular matrix (ECM) [4].

Beyond the widespread applications in tissue engineering, electrospun nanofibers can also be used as a drug delivery system. Since Kenawy et al. first examined the drug release properties from electrospun fibrous mats [5], drug delivery systems based on nanofibers have attracted an increasing interest in the pharmaceutical field. The controlled drug delivery systems are used to improve therapeutic efficacy and safety of drugs by delivering them at a rate dictated by

the need of the physiological environment over a period treatment to the site of action [6]. By using various electrospinning techniques such as conventional, coaxial, and emulsion electrospinning, a number of different drug-loading methods have been developed. For example, drugs can be coated onto/within the nanofibers through exposure of the nanofibers into drug solutions [7,8], embedded within the nanofibers through electrospinning the mixture solution of the polymers and drugs [9,10], or encapsulated within the nanofibers by coaxial [11,12] and emulsion electrospinning [13,14]. However, the methods of coating/embedding drugs onto or within nanofibers generally exhibit apparent initial burst release. The coaxial and emulsion electrospinning approaches allow the formation of core-shell nanofibers which may effectively eliminate the initial burst release and achieve sustained release profiles. Nevertheless, it has been difficult to optimize the coaxial electrospinning conditions. In addition, the emulsifier used in emulsion electrospinning is difficult to remove and may induce biocompatibility issues for

the formed fibers. Development of a new composite nanofiber system allowing for effective loading and sustained release of drugs still remains a great challenge.

Nanotubular structured materials have attracted much attention in the development of drug delivery vehicles with sustained release properties [15-18]. Halloysite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$) is a double-layered aluminosilicate with a chemical structure similar to kaolin. However, morphologically, it has a hollow tubular structure, which is very different from the stacked-plate structure possessed by kaolin [19]. Halloysite is a cheap natural material with a large amount of production [20]. Potential applications of halloysite nanotubes (HNTs) as a drug delivery vehicle were previously reported in literature [20-22], and the drug release rate could be retarded by coating polymers onto the drug-loaded HNTs. Likewise, HNTs are reported to be biocompatible [23]. This suggests that halloysite is a promising drug container. In general, the drug-loaded HNTs is presenting as a formulation of powders or suspensions, which may limit its application in the development of drug-loading devices. It is expected that by combination of the drug loading capability of halloysite and eletrospinning technique, a new drug delivery system with sustained release could be developed for various applications in tissue engineering and pharmaceutical sciences.

In this present work, an antibiotic drug of tetracycline hydrochloride (TCH) was used as a model drug to be encapsulated within HNTs. Then the drug-loaded HNTs were mixed with poly (lactide-co-glycolide) (PLGA) solution for subsequent electrospinning to form composite drug-loaded nanofibers (Figure 1). The HNT-incorporated nanofibers were characterized using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and mechanical measurements. The drug release kinetics of the composite TCH/HNTs/ PLGA nanofibers was studied using UV-vis spectroscopy and compared with drug-loaded HNT powders and electrospun TCH/PLGA blend fibers. Findings from this study are expected to contribute to the rational design of drug delivery systems for various biomedical applications.

2. Experimental section

2.1 Materials

PLGA ($M_w=81,000$ g/mol) with a lactic acid/glycolic acid ratio of 50:50 and TCH (purity >

95%) were purchased from Jinan Daigang Biotechnology Co., Ltd. (China) and Sigma-Aldrich, respectively. Halloysite was a gift from Zhengzhou Jinyangguang China Clays Co., Ltd. (China). Tetrahydrofuran (THF) and N, N-dimethyl formamide (DMF) were from Sinopharm Chemical Reagent Co., Ltd. (China). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from Daikin Industries (Japan).

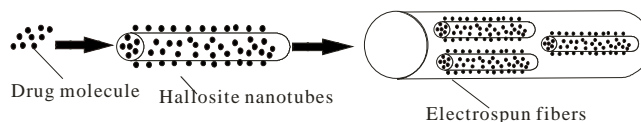


Figure 1 Schematic illustration of drug-loaded electrospun composite nanofibers.

2.2 Preparation of drug-loaded composite nanofibers

TCH (1 mg/mL) was first dissolved in phosphate buffered saline (PBS buffer, pH=7.4) at 80°C for 20 min. The sieved halloysite (via a 45 μm sieve) (1 mg/mL) were mixed with the drug solution. Then, ultrasonic processing was performed for 1 h to make HNTs sufficiently dispersed in the TCH solution. Vacuum (0.085 MPa) was applied to remove the air between and within the hollow tubules. The vacuum was maintained for 12 min. The solution was then taken out from the vacuum and shaken for 5 min. Then vacuum was reapplied to remove the trapped air. Ultimately the cationic drug molecules might be adsorbed onto the negatively charged surface or entrapped within the tubules. The drug-loaded HNTs were dried in an oven for 16 h at 50°C to reach a constant weight. The dried powders were ground down and sieved. The drug-loaded HNTs were then loaded with the drug again by repeating the same process described above. The drug loading efficiency using HNTs was optimized by changing the weight ratio between TCH and HNTs.

To form drug-loaded electrospun composite nanofibers, we first prepared the electrospinning solutions. PLGA with an optimized concentration (10 %, w/v) [24] was dissolved in a mixed solvent of THF/DMF (v/v = 3:1). HNTs (1 wt % relative to PLGA) and drug-loaded HNTs with a loading efficiency of 22.7% (5 wt % relative to PLGA) were blended with PLGA solution for subsequent electrospinning. Moreover, a mixed solution of TCH and PLGA was also prepared. A TCH (20 mg/mL) solution was first prepared by dissolving the TCH powder in HFIP. Then a measured volume of TCH (1

wt % relative to PLGA) solution was added into PLGA solution with continuous stirring for 1 h until a clear and homogeneous solution was obtained.

The electrospinning system used in this study is similar to that used in our previous work [24]. The electrospinning process was carried out under ambient conditions. A fixed electrical potential of 20 kV was employed to charge the steel capillary. The distance between the tip of the needle and the collector was set at 15 cm. The electrospinning solution was fed at a speed of 1.0 mL/h by a syringe pump. Under these conditions, the electrospun PLGA fibers, HNTs/PLGA fibers, TCH/HNTs/PLGA composite fibers, and TCH/PLGA blend fibers were fabricated. Note that the percentage of HNTs, TCH/HNTs, and TCH relative to PLGA in the above 3 cases were 1%, 5%, and 1%, respectively. The PLGA concentration used for fabricating all nanofibers was fixed at 10 wt%. The formed fibrous mats were dried for at least 2 days in vacuum oven at ambient temperature to remove the residual organic solvent and moisture.

2.3 Characterizations

Morphologies of the composite nanofibers were observed under scanning electron microscopy (SEM, JEOL JSM-5600LV, Japan) and field emission scanning electron microscopy (EFSEM, HITACHI S-4800, Japan), respectively. Both of the accelerating voltage was set at 15 kV. Before SEM observations, the samples were sputter-coated with gold films with a thickness of 10 nm, whereas the samples for EFSEM measurements were sputter-coated with carbon films with a thickness of 10 nm. The hollow tubular structure of HNTs was characterized using transmission electron microscopy (TEM, JZM-2100, Japan) at an operating voltage of 200 kV and EFSEM. An aqueous suspension of HNTs (5 μ L) was dropped onto carbon-coated copper grid before TEM measurements. The diameters of the electrospun fibers and the dimensions of the HNTs were measured using Image J software (National Institutes of Health, USA). At least 200 nanofibers or HNTs at different images were analyzed for each sample. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was performed using a Nicolet Nexus670 FTIR spectrometer in a spectral range from 500 to 4000 cm^{-1} . The raw PLGA sample was made by using a film-casting technique. The electrospun composite nanofibrous mats and the raw PLGA film were cut into a round piece with a diameter of 20 mm and then put onto the sample holder before measurements. Zeta potential measurements were performed using a

Zetasizer Nano ZS (Malvern, U.K.) equipped with a standard 633 nm laser. Dilute HNT suspensions (0.1 mg/mL) in aqueous solution containing 1 mM KCl was first prepared. The pH value of the solution was adjusted using 0.1 M HCl or 0.1 M NaOH.

The thickness of the electrospun nanofibrous mats was measured with a micrometer. In order to accurately calculate the porosity, five small strips (10 \times 50 mm²) were cut from the center of the electrospun fibrous mats. The apparent density (ρ_a) and porosity (p) were obtained from the following equations [25]:

$$\rho_a(g/cm^3) = \frac{m(g)}{d(cm) \times s(cm^2)} \quad (1)$$

$$p = \left(1 - \frac{\rho_a(g/cm^3)}{\rho_b(g/cm^3)} \right) \times 100\% \quad (2)$$

Where m , d , and s stand for mass, thickness, and area of the strips, respectively. The bulk density (ρ_b) of PLGA is 1.25 g/cm³.

Tensile test of the electrospun fibrous mats were performed by a material testing machine (H5K-S, Hounsfield, UK) at constant temperature (20°C) and humidity (63%) with a cross-head speed of 10 mm/min. The five strips of the samples (10 \times 50mm²) were tested. From the machine-recorded data, stress-strain (δ - ε) curves of the specimens can be obtained through mathematical conversions:

$$\sigma(MPa) = \frac{P(N)}{10(mm) \times d(mm)} \quad (3)$$

$$\varepsilon = \frac{l}{l_0} \times 100\% \quad (4)$$

Where σ , ε , P , l , and l_0 is the stress, strain, load, extended length, and gauge length, respectively. Breaking strength, failure strain, and Young's modulus were obtained from the strain-stress curves.

2.4 In vitro drug release

To test the drug release from the HNTs, TCH/HNTs powder was weighed out and dispersed into 5 mL phosphate buffer saline (PBS, pH=7.4). Then the dispersed solution was loaded into a dialysis bag and dialyzed against 55 mL PBS buffer. The experiment was done in triplicate. For electrospun nanofiber samples, the TCH/PLGA and TCH/HNTs/PLGA composite nanofibrous mats were both cut into three pieces and their weights were accurately measured. The samples were then placed in different vials

containing 12 mL of PBS buffer. All the samples for release experiments were incubated in a vapor-bathing constant temperature vibrator at 37°C for a period of 30 days. At selected time intervals, a 3-mL solution was taken out from the vials and equal volume of fresh PBS buffer was replenished to them. The release kinetics of TCH was evaluated using an UV-vis spectrophotometer (PerkinElmer Lambda 25, USA). The characteristic absorption peak of 270 nm for TCH was monitored.

3. Results and discussion

3.1 Loading of drug within HNTs

The HNTs used in this study were all sieved and had a uniform tubular structure. SEM and TEM were utilized to characterize the morphology of the HNTs (Figure 2). Relative uniform rod-like structure of HNTs was demonstrated by SEM images (Figure 2a). Some occasional double nanotubular (tube-in-tube) structures are also shown (Figure 2a, marked with a letter “A”). TEM images further verified the existence of the hollow interior of the HNTs (Figure 2b). The measured outer and lumen diameters were estimated to be 75.8 ± 17.5 nm and 17.2 ± 4.6 nm, respectively, and the average length of HNTs was estimated to be 445 ± 256 nm.

The surface potential of the HNTs was tested over a wide range of pH (pH 1.5-12.5). We showed that HNTs were negatively charged (-16 ~ -48 mV) at the studied pH range. The zeta potential values at different pH conditions are all lower than those reported in literature [19]. This could be ascribed to the larger specific surface area of our HNTs having a smaller diameter. The negative charged HNTs could allow the electrostatic attraction of the cationic drug TCH, leading to adsorption of TCH onto the surface and inside the lumen of halloysite when vacuum was applied.

The drug loading efficiency of halloysite was optimized by changing the weight ratio between TCH and HNTs. We found that the maximum loading efficiency could be achieved at the ratio of 1:1. The single loading of halloysite reached an encapsulation efficiency of 23.9%, while the double loading process could reach up to 37% when the total amount of HNTs and TCH was relatively low (e.g., 3 mg for both HNTs and TCH). However, when a relatively large amount of HNTs and TCH were used (e.g., 100 mg), the double loading efficiency decreased to 22.7%. This could be attributable to the fast deposition and aggregation of

the halloysite when vacuum was applied, leading to the existence of relatively more gas in the aggregation and thus less drug loading. The drug-loaded samples used in this study had an encapsulation efficiency of 22.7%.

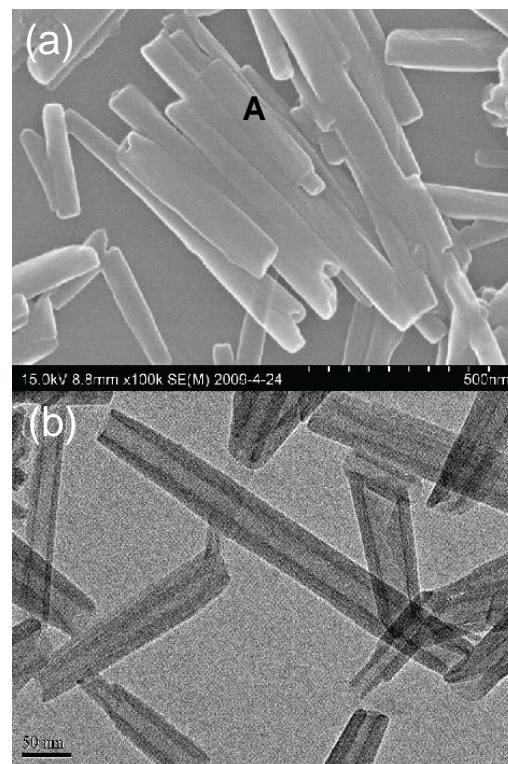


Figure 2 SEM (a) and TEM (b) micrograph of halloysite nanotubes.

3.2 Characterization of electrospun composite nanofibrous mats

The electrospun PLGA fibers, HNTs/PLGA composite fibers, TCH/PLGA blend fibers were fabricated and characterized using SEM (Figure 3). It is clear that the diameter of HNTs/PLGA fibers (Figure 3e) is close to that of pure PLGA fibers (Figure 3d). The morphology of the PLGA fibers does not significantly change after the incorporation of HNTs (Figure 3a,b). However, the diameter of the TCH/PLGA blend fibers (Figure 3c) became much smaller when compared to that of pure PLGA nanofibers. It is generally known that the properties of an electrospinning solution could be significantly affected by an addition of a cationic species. The addition of the cationic TCH drug into the electrospinning solution results in an increase of the surface charge density of the spinning jet [26], which is beneficial for the formation of fibers with a smaller diameter [27].

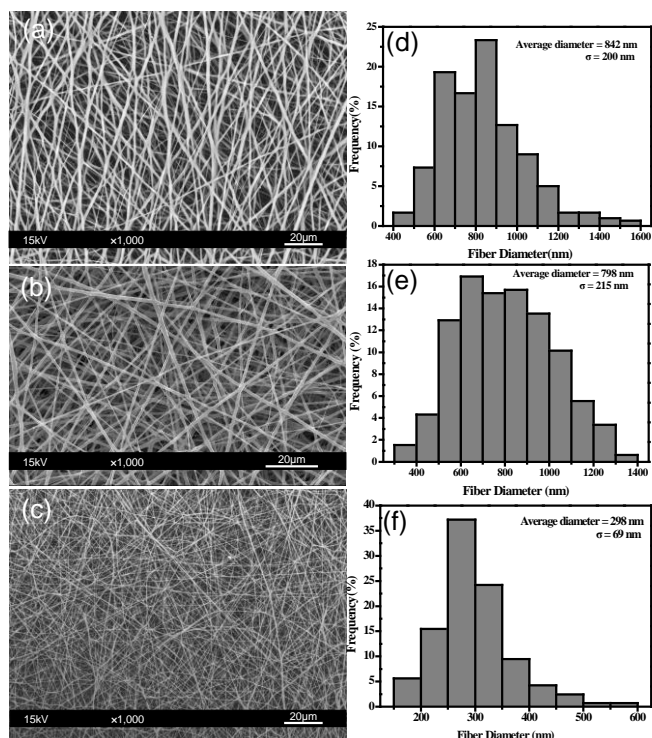


Figure 3 SEM micrographs of the electrospun (a) PLGA fibers, (b) HNTs/PLGA composite fibers, and (c) TCH/PLGA blend fibers. (d), (e), and (f) shows the diameter distribution histogram of the PLGA, HNTs/PLGA, and TCH/PLGA fibers, respectively.

3.3 Porosity and tensile properties

Porosity and tensile properties are important parameters for the application of the electrospun fibers. These porous electrospun fibrous mats can mimic nature extracellular matrix (ECM) for cell attachment, proliferation, and differentiation and can be helpful for drug delivery from the fibers. Likewise, the fibrous mats with a high tensile strength should be required in order for them to be used in practical applications.

The porosities of PLGA and HNTs/PLGA are listed in Table 1. The addition of a small quantity of HNTs (1 wt%, relative to PLGA) does not significantly change the porosity of the mats. However, the mechanical properties of the PLGA fibrous mat were significantly improved. The representative strain-stress curves of the electrospun fibrous mats of PLGA and HNTs/PLGA are shown in Figure 4, and the mechanical parameters are listed in Table 2. Compared with PLGA fibrous mats, the breaking strength, Young's modulus, and failure strain significantly increased with the addition of only a small amount of HNTs (1 wt %), in agreement with literature data [28,29]. The enhancement of the mechanical property of the composite HNTs/PLGA nanofibrous mats should be

ascribed to the alignment of HNTs in parallel to the PLGA fibers (Figure 3b). Therefore, the load can be efficiently transferred from the PLGA matrix to the HNTs.

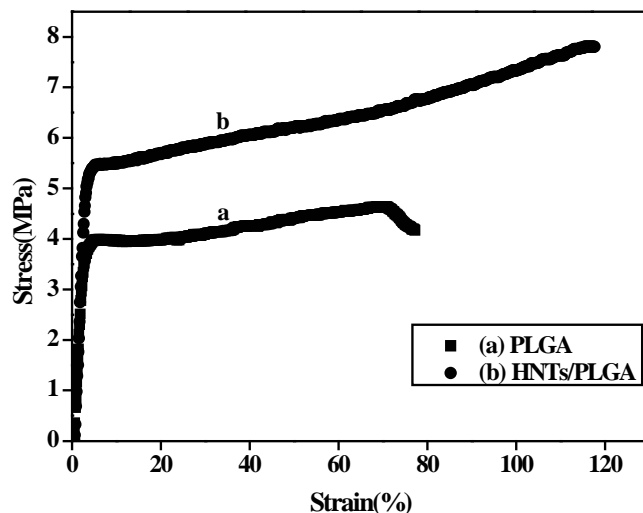


Figure 4 Strain-stress curves of electrospun (a) PLGA and (b) HNTs /PLGA nanofibrous mats.

3.4 ATR-FTIR analysis

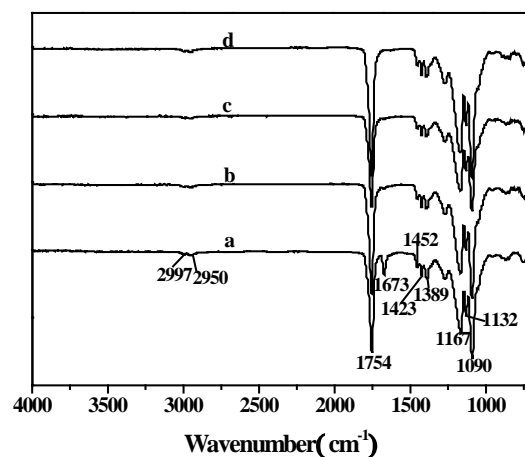


Figure 5 ATR-FTIR spectra of PLGA casting film (a) and electrospun (b) PLGA, (c) HNTs/PLGA, (d) TCH/PLGA nanofibrous mats.

FTIR spectroscopy was used to further characterize the composite nanofibrous mats (Figure 5). It is clear that the FTIR spectra of all electrospun mats are very similar to that of the PLGA casting film, even if HNTs or TCH were incorporated into the fibers. The peaks at around 2997 and 2950 cm⁻¹ were attributed to aliphatic C-H stretching vibrations. Meanwhile, the strong peak at 1754 cm⁻¹ was related to the absorption by an ester carbonyl (C=O) stretch from PLGA. The copolymer PLGA also had characteristic peaks at 1452 cm⁻¹

(methyl group C-H stretching), two peaks at 1423 and 1389 cm^{-1} (wagging vibrations from saturated C-H bonds), and C-O peaks at 1271, 1167, 1132, 1090 and 1052 cm^{-1} [24,30]. The peak at 1673 cm^{-1} for PLGA casting film (Figure 5, curve a) may be caused by the C=O stretching vibration from amide bands of the

residual solvent DMF. However, this peak was not prominent in the spectra of all electrospun nanofibers. This should be due to the large surface area of the nanofiber structures, allowing easy evaporation of the solvent.

Table 1 Apparent density and porosity of electrospun PLGA and HNTs /PLGA nanofibrous mats. Data are representative of independent experiments and all data are given as means \pm SD ($n=5$).

Sample	Apparent density (g/cm^3)	Porosity (%)
PLGA	0.307 ± 0.007	75.48 ± 0.56
HNTs/PLGA	0.316 ± 0.019	74.75 ± 1.49

Table 2 Tensile properties of electrospun PLGA and HNTs/PLGA nanofibrous mats under the same processing conditions. Data are representative of independent experiments and all data are given as means \pm SD ($n=5$).

Sample	Breaking Strength (MPa)	Failure Strain (%)	Young's Modulus (MPa)
PLGA	4.21 ± 0.35	76.15 ± 8.38	118.8 ± 10.5
HNTs/PLGA	6.61 ± 1.66	93.6 ± 21.0	141.8 ± 6.7

3.5 Release properties of drug-loaded composite nanofibrous mats

The release profiles of TCH from TCH/HNTs powders, electrospun TCH/PLGA and TCH/HNTs/PLGA nanofibrous mats are shown in Figure 6. TCH embedded in the PLGA nanofibers formed by electrospinning the mixture solution of PLGA and TCH exhibited an obvious initial burst release. Within the first 24 h, 83.8 % of the drug was released. The drug release reached a plateau after 48 h. While both drug-loaded TCH/HNTs powders and TCH/HNTs/PLGA fibrous mats had a sustained release within a month, with only 36 % and 32 % of TCH released after 28 days, respectively. The release of TCH from TCH/HNTs powders is slower than that reported by Kelly et al [22]. In their work, they showed that 88 % of TCH was released at day 9. The slower release of TCH from HNTs in our study could be due to the fact that the specific surface area of HNTs used in our study is much larger than that they used [19]. The HNTs they used had typical dimensions of 2-3 μm length and 0.3/0.1 μm outer/inner diameter, which are much bigger than those used in our current study. The TCH/HNTs powders encapsulated within the PLGA nanofibers further slowed down the release of TCH. This suggests that the PLGA nanofibers can be used to hinder the release of TCH from the HNTs to the solution. More importantly, after incorporated within the nanofibrous mats, the TCH/HNTs could be formulated as a drug-containing scaffolding material for both tissue engineering and sustained drug delivery.

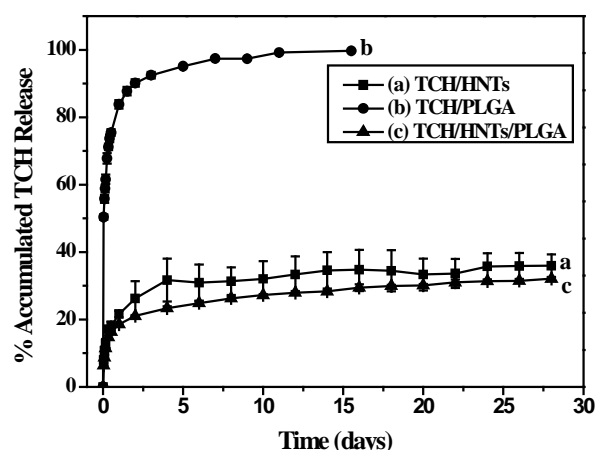


Figure 6 In vitro release of TCH from (a) drug loaded TCH/HNTs powders and electrospun (b) TCH/PLGA and (c) TCH/HNTs/PLGA nanofibrous mats. The samples were incubated in PBS buffer (pH=7.4) at 37°C.

4. Conclusion

In summary, we have successfully fabricated electrospun HNT-containing composite polymer nanofibers for efficient drug loading and sustained release. The incorporation of drug-loaded HNTs not only significantly improved the mechanical properties of the fibrous mats, but also prolonged the release rate of the drug, appreciably eliminating the initial burst release characteristics. We show that the addition of HNTs does not change the uniformity of the

electrospun PLGA nanofibers. The prolonged release profile achieved using the double encapsulation of drugs within HNTs and PLGA could be extended for encapsulation and release of other drugs. With the excellent biocompatibility of HNTs and PLGA polymers, the developed system should be amendable for the applications in tissue engineering and pharmaceutical sciences.

Acknowledgements

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Electromagnetic Shielding Characterisation of Several Conductive Fabrics for Medical Applications

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Abstract: This paper evaluates and compares the electromagnetic (EM) shielding of a diverse range of conductive fabrics in order to analyse their suitability for use in wearable medical applications. The Shielding Effectiveness (SE) was characterised in terms of fabric structures, conductive materials, mass, thickness and washing durability. Experiments were carried out on single and double layers of fabrics using broad frequency range and SE was measured using different methodologies. EM shielding is the process of limiting the flow of EM fields between two locations by a barrier. The shielding barrier needs to have high conductivity, dielectric constant or high magnetic permeability, and the shielding happens due to reflection, absorption or multiple reflections of the incident radiation by the barrier. Therefore, shielding is important to block electromagnetic radiation that could be harmful to electronic devices, environment and humans.

Keywords: electromagnetic interference; shielding effectiveness; conductive fabrics; medical textiles.

1. Introduction

Textiles have been highly considered in applications for electromagnetic interference (EMI) shielding in the electrical & electronic industries as well as for the production of protective garments due to the increasing concern about health issues caused by human exposure to radiation. The emerging role of textiles in EMI shielding is mainly due to their desirable properties in terms of flexibility, versatility, low mass and low cost. Textiles are intrinsically non EMI shielding materials and are rather insulating materials; however, they can successfully turn to be EMI shielding after raw-material changes, new production process or process adaptations that can make them electrically conductive [1].

Some of the methods to obtain conductive fabrics are fibres and yarns made of copper, aluminium, stainless steel, intrinsically conductive polymers (ICP), and/or metallic fillers or coatings incorporated in the yarn production. These processes are based on mixing the fibre polymer with metal fillers during the chemical processes such as melt or wet spinning; or by twisting and wrapping a synthetic fibre with metallic yarns using mechanical spinning processes. These technologies are less often used due to their inherent complexities [2-20].

Other approaches include the application of conductive materials on the surface of the fabric itself using lamination, coating, spraying, ionic plating, electroless plating, vacuum metallisation, cathode

sputtering, and chemical vapour deposition. Coating usually does not change the flexibility of the fabrics and is applied in very thin layer, low mass and closed fabrics. When coating is applied during the yarn production, it is possible to obtain small diameter conductive yarns, and therefore, very flexible and light weight fabrics. Most of the conductive fabrics in the market made by coating technologies have very homogeneous and closed structures thus exhibiting high EMI shielding capabilities and isotropic behaviour. [21-41].

Conductive fabrics can also be made of metallic yarns (i.e. stainless steel, copper); however, they are difficult to process. These types of yarns tend to have low flexibility due to their large diameter, which produces a heavier and uncomfortable fabric. To reduce this effect, metallic yarns are used to replace a few synthetic yarns in the structure, thereby reducing the overall stiffness of the fabric. There are several research works using this method, where the fabrics are analysed having diverse fabric constructions, different densities, patterns, yarn diameters, quantity of conductive yarns in the structure, layers and yarn direction [2-14,17-19,33,42-48].

1.1 Shielding Effectiveness (SE) measurement

The shielding effectiveness characterisation is usually evaluated by coaxial transmission line methods, waveguide methods and open space methods. However, there is a lack of generally accepted and official

standard methods for measuring shielding effectiveness of fabrics. The following methods are normally used for research work and quality control purposes [49].

The “ASTM D 4935-99- coaxial transmission line method for planar materials” is the most common and easiest to use. The shielding effectiveness measurements are normally carried out from 30 MHz to 1.5 GHz. The measurement device consists of a network analyser, which is capable of measuring incident, transmitted and reflected powers, and a sample holder. The shielding effectiveness is determined by comparing the difference in attenuation of a reference sample to the test sample, taking into account the incident and transmitted power. This method can be applied assuming the following prerequisites: the measurements obtained pertain to the far-field (plane wave) and the thickness of the tested materials cannot exceed 1/100 of the wavelength of the EM wave in open space.

The “IEEE-STD 299-2006” (replaced the cancelled “MIL-Standard 285”) is probably the most frequently referenced standard covering attenuation measurements for shielded enclosures within the frequency range of 100 kHz to 10 GHz. There are numerous adaptations of this method which have been devised to evaluate the properties of flat shielding materials. Measuring shielding effectiveness using this test setup is time-consuming and troublesome. It requires excellent proficiency and measurement experience. It consists of a transmitting and receiving antenna, a network analyser or similar equipments and an anechoic chamber with a probe window where the fabric is placed.

Some less used methods are: “ASTM E7-83 coaxial transmission line method” and open space methods. These methods are not used commonly due to their complexity. The first one requires a network analyzer and a coaxial transmission-line cell, whereas the second one requires a network analyser, transmitting and receiving antennas.

2. Experimental work

In this study, the evaluation of the EM shielding fabrics was made by measuring the Shielding Effectiveness (SE). SE is expressed in decibels (dB) and is a logarithmic representation of a ratio measurement. It is most commonly used for expressing power ratio at high frequencies (Eq. 1), where P1 is the transmitted EM power with the fabric and the P2 is the transmitted EM power without the fabric, or P2 can also be assumed as the total incident EM power itself.

$$SE [dB] = 10 \log (P1/P2) \quad (1)$$

2.1 Materials

The tested fabrics were knitted, woven, and nonwoven nylon and polyester fabrics, and were coated with metals, conductive polymers, or made by carbon fibres. Coated fabrics were chosen as they are isotropic, have high shielding effectiveness, high flexibility and low mass. The fabrics and their characteristics are summarised in Table 1.

Table 1 List of fabrics

Fabric	Material	Weight	Thickness
		[g/m ²]	[mm]
1 Nonwoven “Bonn”	Ag	na	0.235
2 Woven ”Berlin”	Pu Ag	60	0.114
3 Woven “Nora Dell”	NiCuAg	95	0.130
4 Woven “Zell”	SnCuAg	72	0.073
5 Nonwoven	Carbon	34	0.640
6 Knit	Ag	40	0.250
7 Woven “Zelt”	SnCu	72	0.064
8 Woven “Flectron”	Cu	80	0.152
9 Stretch Knit	Ag	130	0.500
10 Nonwoven "SPB15"	Ag	62	0.240
11 Knit "STUL35"	Ag	35	0.250
12 Nonwoven	Carbon	12	0.150
13 Mesh “Eeontex”	Ppy	72	na
14 Woven "TCS72T"	SnCuAg	72	0.076
15 Woven "SBRM48"	Ag	48	0.114
16 Woven "CSR68"	CuAg	68	0.076
17 Woven “Eeontex”	Ppy	223	0.500
18 Nonwoven“Eeontex”	Ppy	na	0.600
19 Woven“ShielditSuper”	NiCu	230	0.170

2.2 Shielding Effectiveness methods

The shielding effectiveness was determined using two methods, one for measurements up to 1 GHz and another from 1 GHz to 6 GHz.

2.2.1 Method under 1 GHz

The standard method ASTM D 4935 was used. The fabrics were tested in a flat configuration, with the same direction and side in relation to the sample holder. The shielding effectiveness was determined by comparing the difference in attenuation of a reference sample to the test sample, taking into account the incident and transmitted radiations.

2.2.2 Method above 1 GHz

The fabrics were tested using the open space method, up to 6 GHz, using a transmitting and receiving horn antenna and a set up including a network analyser, amplifier and absorbing foam to avoid diffraction. Not all fabrics were able to be tested due to the high size of the sample needed and the low dynamic range, which permitted only fabrics with less than 85 dB of SE to be tested.

3. Results and discussion

3.1 EM shielding results

The two methods used showed similar results. The fabrics maintained basically the same SE when at both low and high frequencies. The SE results of less than 1 GHz are found in Figure 2, whereas results from 2 GHz to 6 GHz are depicted in Figure 1.

3.2 EM shielding results in relation to fabric structure

The SE results showed that metal coated woven fabrics have the highest SE, usually > 70 dB. Woven structures have a very closed and tight structure, thus having very little space between the yarns, and a very flat and homogenous surface.

Metal coated nonwovens have SE about 50-60 dB, whereas carbon fibre nonwovens have $SE < 40$ dB. The nonwoven structure is usually very light and loose; therefore, having greater space openness between fibres.

Metal coated knitted fabrics have lower SE, about 40 dB, due to the open space structure and flexibility of the fabric, therefore, allowing EM radiation to pass through the structure at shorter wavelengths.

All the polypyrrole (Ppy) coated fabrics tested showed a $SE \leq 20$ dB, which is more due to the poor conductivity of polypyrrole rather than the fabric's structure itself. The polypyrrole coated mesh tested (fabric number 13) showed the lowest SE, which is also related to the highly open structure.

All conductive coated fabrics showed similar behaviour within the frequency range tested, possibly because of their homogeneity and isotropic structure.

3.3 Absorption and reflection behaviour of EM shielding

During the SE measurements it was also possible to obtain the value of the reflected radiation (also known as Return Loss or S11 parameter) from the sample under test. It was straightforward to calculate the absorbed radiation by the fabric, once the transmitted radiation is known by the SE results.

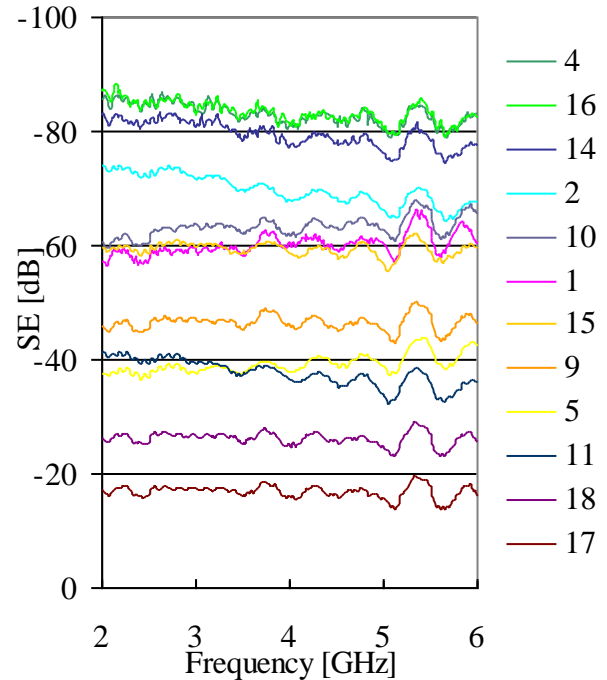


Figure 1 SE results above 1 GHz.

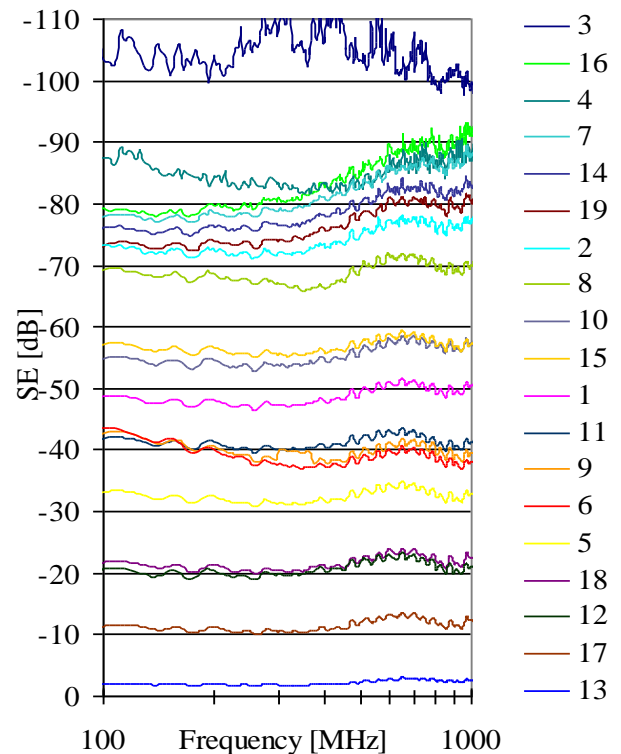


Figure 2 SE results under 1 GHz.

Results obtained in decibels can be transformed into percentage, making it easier to understand which part of radiation is reflected, absorbed and transmitted by the fabric (Table 2).

Table 2 Percentage of SE

SE [dB]	SE [%]	SE [dB]	SE [%]
1	20.6	50	99.999
5	68.4	60	99.9999
10	90.0	70	99.99999
20	99.0	80	99.999999
30	99.9	90	99.9999999
40	99.99	100	99.99999999

The percentage of SE was calculated using Eq. 2, the percentage of transmission (T), reflection (R) and absorption (A) by Eq. 3, Eq. 4 and Eq. 5 respectively, and P3 of Eq. 4 relates to the EM power that is reflected by the fabric.

$$SE [\%] = 100 - (P1/P2) \cdot 100 \quad (2)$$

$$T [\%] = (P1/P2) \cdot 100 \quad (3)$$

$$R [\%] = (P3/P2) \cdot 100 \quad (4)$$

$$A [\%] = 100 - T [\%] - R [\%] \quad (5)$$

Conductive materials have different absorption and reflection behaviours: metals are known for having high reflectivity; whereas carbon fibre and conductive polymers are known for having high absorption behaviour. This trend was also observed in the results obtained.

Knitted fabrics, carbon fibre nonwovens, heavier and polypyrrole coated fabrics showed higher absorbance; however, it was not a standard behaviour for all the fabrics analysed and it highly fluctuated within the frequency spectrum tested.

The reflection (Return Loss) of most of the fabrics at 500 MHz varied from 1.5 dB to 2.5 dB, which meant the fabrics reflected between 55% and 75% of the incident radiation, and thus, they absorbed between 25% and 45% of the incident radiation, since the transmitted radiation through the fabric was considered to be 0%.

The fabrics tested were relatively thin, and most of them had very low resistivity, which meant that the obtained absorption percentage was likely to be due to multiple reflections within the internal fibres rather than actual absorbance.

Results also showed that the absorption increased with higher frequency up to 1 GHz (± 1 dB, $\pm 20\%$), and then reduced at 1.5 GHz ($\pm 5\%$), while highly fluctuated within the frequency spectrum tested.

Some fabrics were tested in double layers in order to better understand the relation with SE and thickness. The double layers did not show a significant change in percentage of absorption, which confirmed that the absorption is not related to thickness, it is rather related to the dielectric properties of fibres and their structure configuration.

3.4 Conductive materials used for EM shielding

The metals normally used for coating are: Ag, Sn, Cu, Ni. Each one has its own properties: Ni and Sn are usually coated after copper as it prevents corrosion and helps to provide mechanical protection; Ag is usually the first metal coated and this is then coated with other metals to obtain better properties.

Fabrics with more than one metal coated usually have higher SE, and metal coating has higher SE than coatings from Polypyrrole (Ppy) or carbon fibres. However, the quantity of material used, type of coating and structural homogeneity also influences the SE.

The optical microscope images showed that all of the fabrics used were coated after their construction, which produced a conductive path on the surface of the fabric. The surface coating was very homogenous and also penetrated within the internal fibres in some parts. With this type of coating, any change in the structure such as stretch and friction could cause damage to the conductive path of the surface.

3.5 Durability of EM shielding after washing

Washing tests were undertaken in order to evaluate further the SE. The washing cycles were performed using a washing machine at two conditions:

A: 5 cycles of 45 minutes at room temperature with washing powder, and hang dried also at room temperature after each cycle.

B: 1 cycle of 90 minutes at 40 Celsius degrees with washing powder, and hang dried at room temperature.

Condition "A" was performed in all of the fabrics, whereas, condition "B" was performed only in fabrics having different metallic coatings. Ag and Ni coatings were not visually damaged after washing treatments. Cu and Tin coatings were highly damaged after hot washing, as well as the polypyrrole coating. Fabrics number 14 and 16 can be observed in Figure 3 and Figure 4, after hot washing.

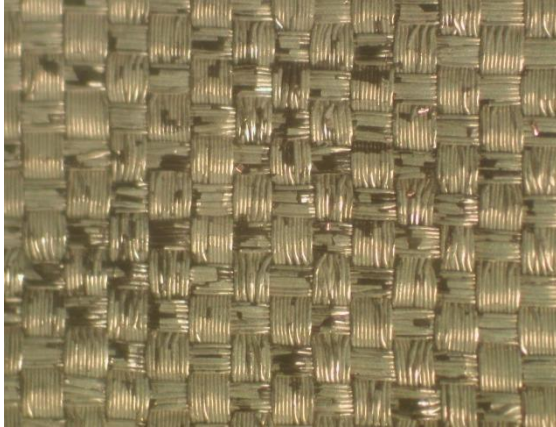


Figure 3 Fabric number 14 after hot washing

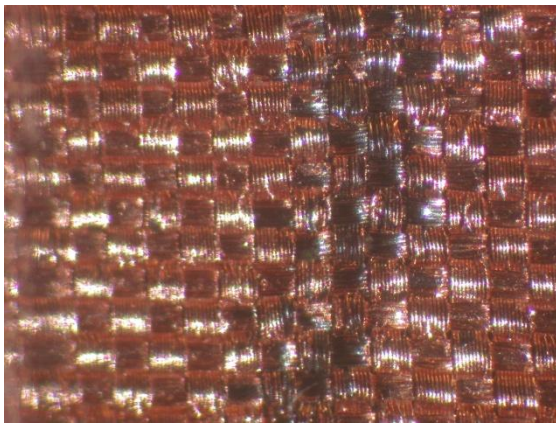


Figure 4 Fabric number 16 after hot washing.

The SE after washings was not significantly affected in fabrics coated with Ag. On the other hand, the SE after washings was reduced on fabrics coated with Sn, Cu and Ni: ± 15 dB less after cold washes and ± 30 dB less after hot washing. It was not a drastic reduction of performance observed as the fabrics had very high SE and 30 dB less represents ± 0.01 % less in fabrics with $> 99.9\%$ of attenuation. Ag coated fabrics number 10, 11 and 15 are showed in Figure 5, 6 and 7 respectively. Whereas fabrics number 3, 14 and 17 are illustrated in Figure 8, 9 and 10 respectively, where it can be seen the high influence of the washing treatments in SE. In the figures, the green lines represents the original fabric, the blue lines the washing condition “A”, and the red lines washing condition “B”.

The SE values after washing were highly reduced with Ppy coated fabrics: ± 10 dB less, which represents a reduction of $\pm 20\%$ in attenuation in the case of low SE fabrics.

Fabrics made of carbon fibres were not possible to wash because of the very light and fragile structure. These fabrics were not coated, instead, the carbon fibres itself were used as component within the

structure. Therefore, the SE behaviour believed not to have been affected after the washing treatments.

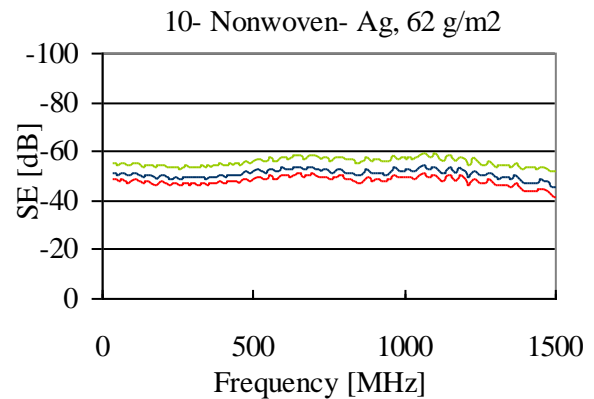


Figure 5 SE results after washing treatments.

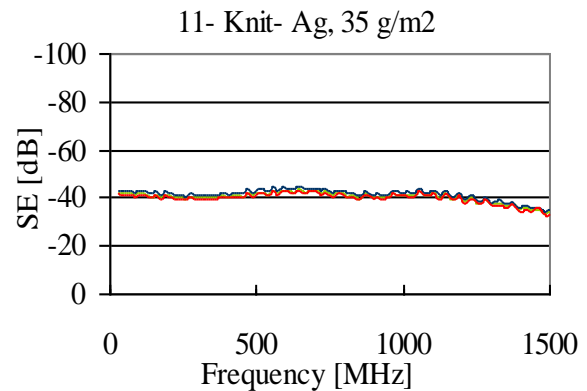


Figure 6 SE results after washing treatments.

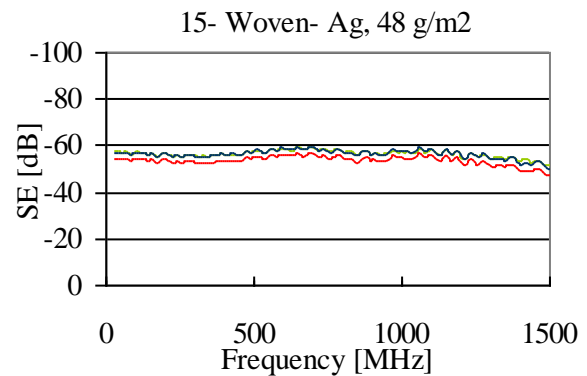


Figure 7 SE results after washing treatments.

3.6 EM shielding in relation to thickness of fabrics

In the case of the two carbon fibre nonwovens tested, the results showed that a thicker and heavier fabric had higher SE (± 10 dB on ± 20 g/m² of higher mass). On the other hand, the different Ag coated knitted fabrics tested did not show a significant change in SE because

of the mass. In addition, also similar woven fabrics tested did not show the same SE results.

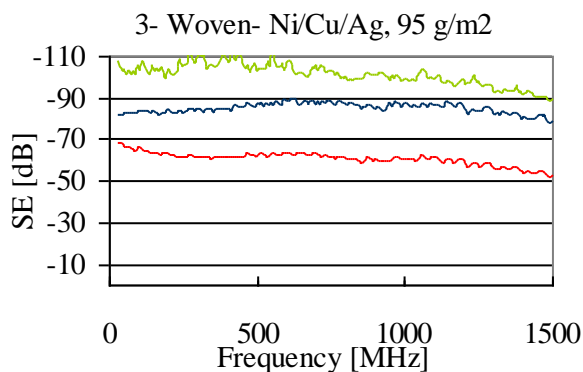


Figure 8 SE results after washing treatments.

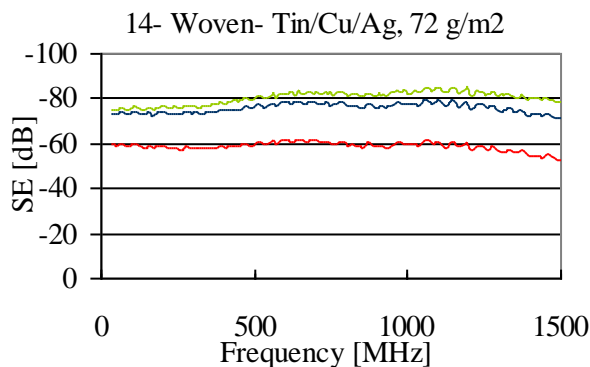


Figure 9 SE results after washing treatments.

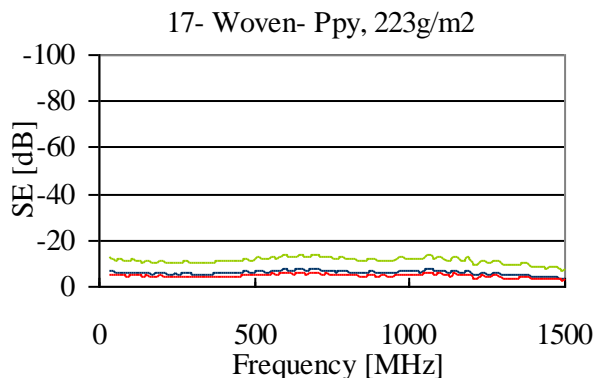


Figure 10 SE results after washing treatments.

Some fabrics were tested in double layers; they showed a slight increase in SE values which was likely related to less free space present in the fabric.

The SE values of knitted fabrics (number 11) increased around 10 dB; on nonwoven fabrics (numbers 12 and 10) around 10 dB and 20 dB respectively; on woven fabrics (number 8 and 15) about 10 dB; and on the low SE fabrics coated with polypyrrole (number 13 and 17) increased about 2 dB and 5 dB. The increase on

SE values had more impact on low SE fabrics than on high SE fabrics (Table 3).

Table 3 SE results at 1GHz

Fabric	[dB]	[dB]	[%]	[%]
	single	double	single	double
8	70	80	99.99999	99.999999
10	60	80	99.9999	99.999999
15	60	70	99.9999	99.99999
11	40	45	99.99	99.997
12	20	30	99	99.9
17	12	17	94	98
13	2.5	4.5	44	65

4. Conclusions

All fabrics tested exhibited more than 99% electromagnetic shielding capabilities, apart from fabrics 13 and 17, which meant that they block the radiation almost totally in original and flat condition up to 6 GHz.

Most fabrics tested blocked the radiation after 5 cold and 1 hot washes; however, the metal coating properties begun to deteriorate due to damage caused during repeated cycles. Ag coated fabrics were the only ones not damaged after hot washing.

Therefore, the best fabrics for wearable medical applications considering cost, durability in terms of washing, flexibility, health, and low mass are fabrics number: 1, 10, 11, 12, and 15.

These fabrics were coated with Ag, which is already used in the textile field and has also well known antimicrobial and anti-odour properties. Fabric number 12 was the only one not coated with any metal or conductive polymer, but made of carbon fibres. This fabric was not washed due to the high fragility and low mass characteristics. It was chosen to be evaluated during further application tests.

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Biodegradability of Flax Noil Fibers Reinforced Poly(Lactic Acid) Composites

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Abstract: The composites of poly(lactic acid) (PLA) with untreated or alkali treated (A-) or silane-coupling treated (SC-) flax noil fibers (flax) were prepared using non-woven method and hot pressing technology. The biodegradability of the composites was evaluated by activated soil-burial test. The presence of untreated flax or A-flax or SC-flax led to the acceleration of weight loss due to preferential degradation of flax, which was shown by the SEM micrographs and FTIR spectra. Rates of weight loss decreased in the order flax/PLA (24.0%/35days) > A-flax/PLA (20.6%/35days) > SC-flax/PLA (17.8%/35days) and decreased with interface shear strengths of the composites. The weight losses of PLA and flax after 35 days are 4% and 52.5% respectively.

Keywords: biodegradability; poly (lactic acid) (PLA); flax; non-woven method; interface property

1. Introduction

Natural flax noil fibers offer good opportunities as reinforcement materials for composites attributing to its advantages, such as renewability, high specific properties, low cost, biodegradability, non-hazardous nature and so on [1-5]. For the purpose of making completely biodegradable green composite, biodegradable polymers has been chosen as matrix. Among all kinds of biodegradable polymers, PLA seem to score well on all the necessary properties: the density is low; degradation behavior, mechanical properties and glass transition temperatures are acceptable and their melt points are almost ideal in producing flax fiber reinforced composites; above all, PLA is not quite expensive and already commercially available [6].

Some researchers have already identified the possibilities for biodegradable composite products by combining PLA with flax fibers [7-10]. In order to improve the interfacial bonding of the composite, S. Goutianos[9] and R. A. Shanks [10] adopted alkali treatment to treat the fibers. Although biodegradability is one of the most important properties of the bio-composites, little was reported on this aspect of the composite.

Cao Y et al [12] performed the biodegradability test in a series of plastic boxes containing characterized soil, which was 1:1(mass ratio) mixture of red gravel soil and leaf mold for gardening. The pH of the soil was about 7 and each specimen was buried at a depth of

8cm from the surface in the soil. Cao Y et al [12] found that the addition of bagasse fiber to the polycaprolactone composite caused the acceleration of weight loss as a result of the preferential biodegradation of fiber. The weight loss increased with the increase of the fiber content. Wu CS et al. [13] did the biodegradability test in soil environment. Five samples were weighed and then buried in boxes of alluvial-type soil, obtained in May 2005 from farmland topsoil before planting. The soil was sifted to remove large clumps and plant debris. The Soil was maintained at ~20% moisture in weight and samples were buried at a depth of 15 cm. Wu CS et al. [13] found the biodegradation of the poly(3-hydroxybutyric acid) composite increased with the increase of the wood fiber.

In our previous reports, we have revealed that the mechanical properties of the flax noil fibers (flax) reinforced PLA (flax/PLA) composites are quite promising compared with flax/PP composites, which has been used as automotive interiors recently [7,11]. In this paper the alkali and silane-coupling reagents were used so as to improve the interface bonding property of the composites. The objective of this work is to study the effect of fiber content and interface bonding property on the biodegradability of the composites, which will be used to build the biodegradability prediction model in the future.

2. Experimental

2.1 Materials

A commercially available PLA fiber is used as the polymeric matrix. The PLA fibers are in length of 38mm, melting point of 168°C. Flax noil fibers are used as the reinforcement, whose diameter ranges from 15 to 155µm. The average length, diameter and tensile strength of flax are 71.3mm, 63.89µm and 417.57MPa respectively.

2.2 Surface modification

Flax noil fibers are initially treated before mixing with PLA fibers. The fibers are firstly opened by the opener so as to wipe off the impurities, over-length & under-length fibers and improve the orderliness level of the length and fineness of the fibers. Then the fibers are immersed into the 1% alkali solution at room temperature for 1h, followed by washing with distilled water until no sodium hydroxide is left. Subsequently, the fibers are dried at 80°C for 2h followed by drying to constant weight in air.

As far as silane-coupling treatment, the fibers are soaked in 1% $\text{H}_2\text{N}(\text{CH}_2)_3\text{Si}(\text{OC}_2\text{H}_5)_3$ solution for 2h, then being dried at 80°C for 1.5h followed by being dried in the air to constant weight.

The flax fibers with no treatment, alkali treatment (A-) and silane-coupling treatment (SC-) are prepared separately before performing the composite.

With the addition of 45 w.t.% flax the PLA composite showed the best mechanical properties according to our earlier results[11], by which this fraction of flax is selected to perform the composites with surface treatment.

2.3 Composite preparation

The flax and PLA fibers are mixed together by non-woven technology. First, the pulling fibers are opened and scotched by the opener and then the mixture is carded by the carding machine in order to make the even fiber web. Second, the fiber web was piled up longitudinally so as to make the preform with the density of 1400g/m³ and fibers weight fraction of 40, 45, 50, 60% respectively. Third, the preform is molded into composite at a temperature of 190°C, pressure of 12.5MPa and time period of 20min.

2.4 Characterization

Biodegradability of the composites is determined by measuring the weight loss of the thin-plate specimens

[12-13]. The specimens are buried in the activated soil (soil) provided by Tianjin Wastewater Treatment Plant. The microorganism content of the soil is 2g/L. According to ISO 14852:1999(E) [14], the solid concentration of the soil is 20g/L. In order to maintain the concentration, the container of the soil is sealed with black plastic bag.

The samples are vertically buried in the soil at a depth of 10cm. The plastic web bags are used to hold the samples in the liquid soil, followed by being picked out from the soil after 5, 10, 15, 20, 25, 30, 35days, respectively. The specimens are picked out from the bag and washed with distilled water, followed by being dried to a constant weight at 80°C in an oven.

Digital pictures of the specimens are taken by a digital camera (Canon PC 1192, Canon, Japan).

The morphology of the specimen surface is observed by a scanning electron microscope (SEM) (KYKY 2800, the Research and Development Center of Scientific Instruments of Chinese Academy of Science, China). Prior to the observation, the specimens are coated with silver so as to achieve optimal imaging.

The FTIR of the specimens are taken by the Fourier transform infrared spectrometer (Vektor 2.2, Bruker, Germany).

3. Results and discussion

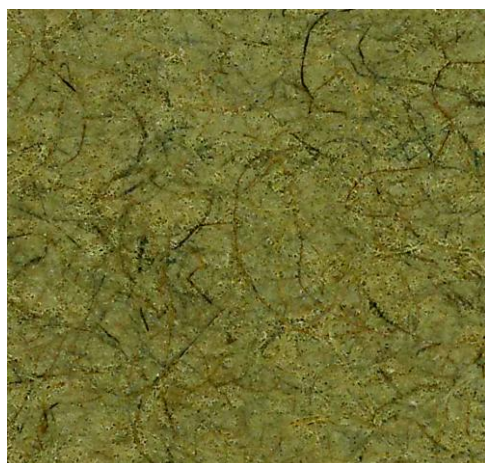
3.1 Composite surface

Figure 1 shows the surfaces of 45flax/PLA (the weight fraction of the flax is 45%) before and after biodegradation of 35 days. It can be seen that there is a significant difference between Figure 1(a) and Figure 1 (b). The 45flax/PLA buried for 35 days could not be fully recovered because of a considerable fragmentation. There are lots of cavities on the surface of the composite, by which it can be seen the biodegradation has occurred on the surface of the composite.

3.2 Effect of fiber content on the biodegradability of composites

Figure 2 shows that the weight loss increases with burial-period for all the samples. It can be seen the flax has a much higher biodegradability than PLA. The biodegradability of the composites increase with the fiber content, which agrees well with the results reported by Yong Cao [12] and Chin-San Wu [13]. It is marked that the weight loss of 60flax/PLA (42.1%) is about twice as much as 40flax/PLA (20.6%). As shown

in Figure 3, the SEM micrographs of 60flax/PLA after 35days burial shows more hollows and gaps than 40flax/PLA. Based on the phenomena above we could conclude that the biodegradation of flax is the main reason for the biodegradation of flax/PLA, which can be also supported by the results of Teramoto N [15].



(a) Before biodegradation



(b) After biodegradation

Figure 1 45flax/PLA surface before and after biodegradation of 35 days.

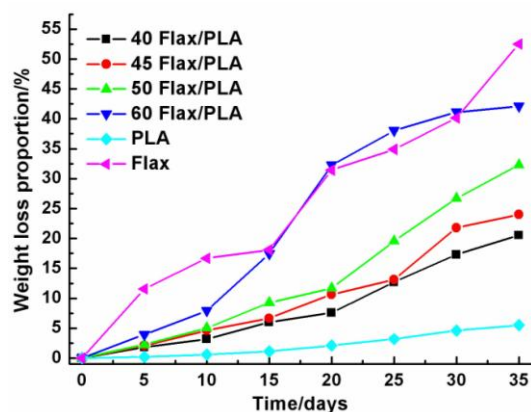
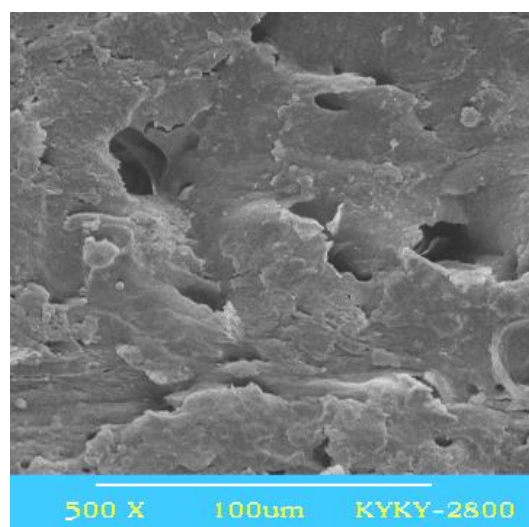
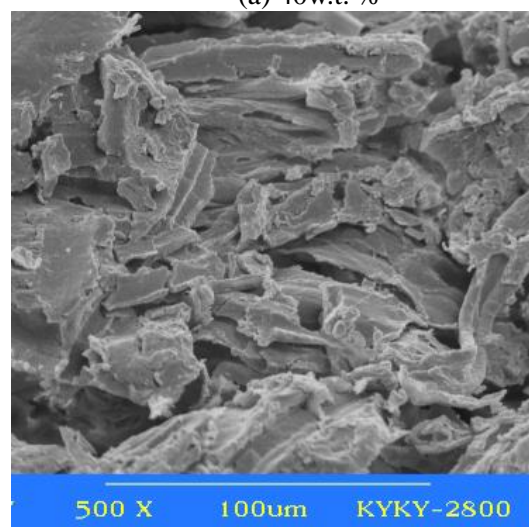


Figure 2 Weight loss data of the specimens of composite, flax fibers and PLA.



(a) 40wt. %

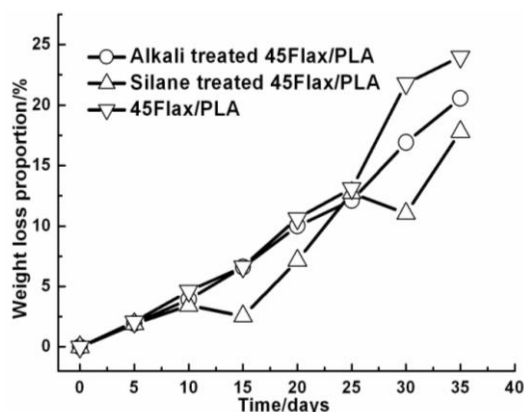


(b) 60 wt. %

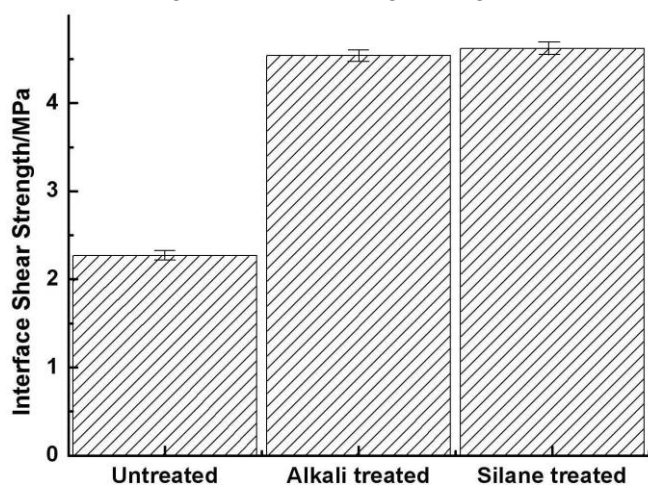
Figure 3 SEM micrographs of the composites with different fiber content.

3.3 Effect of interface property on the biodegradability of composites

As shown in Figure 4 (a), rates of weight loss decreased in the order of 45flax/PLA (24.0%/35days)>A-45flax/PLA(20.6%/35days)>SC-45 flax/PLA(17.8%/35days). As far as interface shear strength, as shown in Figure 4 (b), the order is reverse, which is 45flax/PLA (2.27 MPa) < A-45flax/PLA (4.54 MPa) < SC-45flax/PLA (4.62 MPa). This means the interface bonding property of the composite is in negative correlation with its biodegradability.



(a) Weight loss data during biodegradation

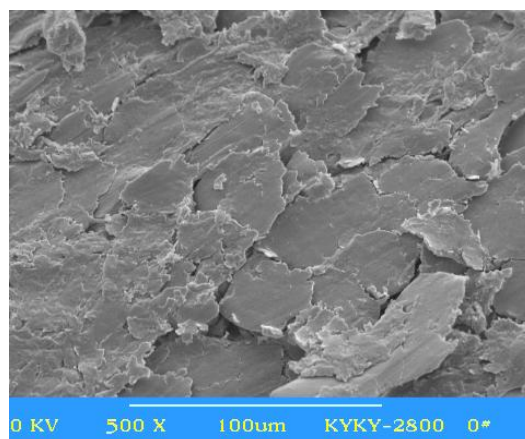


(b) Interface shear property

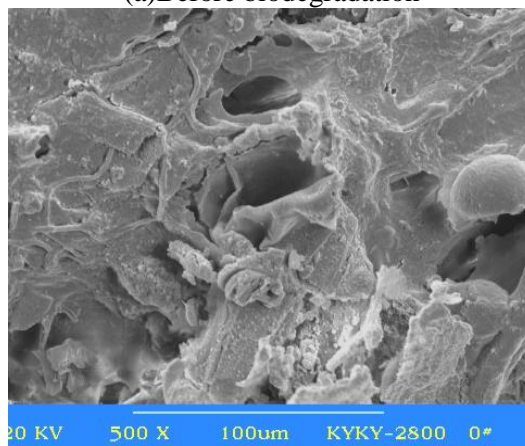
Figure 4 Properties of the composites before and after surface treatments.

As shown in Figure 5 (a) there are gaps between the fibers and matrix, while Figure 5 (b) shows after biodegradation the matrix breaks from the composite as a result of the accumulation of fibers biodegradation. As shown in Figure 6 (a) the shoulders at 3328.81cm^{-1} and 1756.38cm^{-1} are associated with the hydroxyl band of flax fibers and carbonyl band of PLA matrix respectively. It can be seen the flax fibers biodegrades from the composite shown by Figure 6 (a) and (b), and the sharp decrease at 1756.38cm^{-1} in Figure 6 (a) indicates the biodegradation of the PLA in the flax/PLA.

Because the effective interface between the flax fibers and microbes size getting smaller with the increase of interface shear strength, the composite with higher interface shear strength is equipped with lower biodegradability.

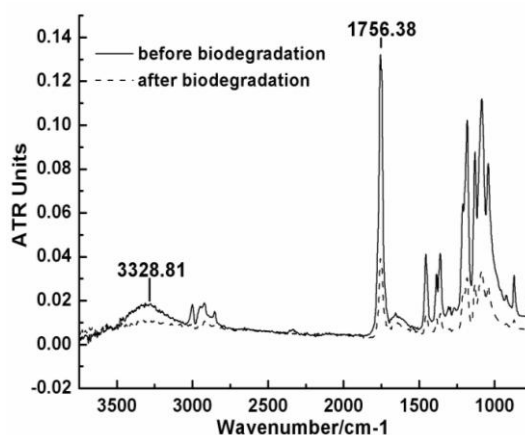


(a) Before biodegradation

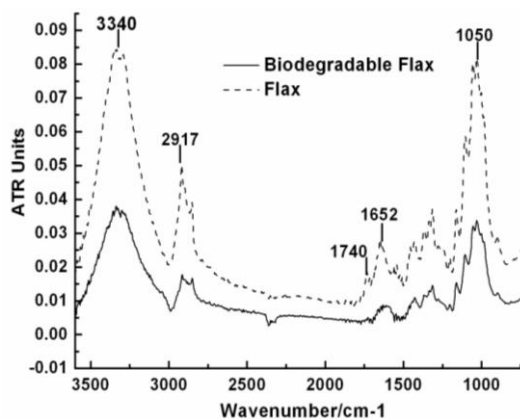


(b) After biodegradation

Figure 5 SEMs of 45flax/PLA before and after biodegradation.



(a) 45flax/PLA



(b) flax fibers

Figure 6 FTIR of 45flax/PLA and flax before and after biodegradation.

4. Conclusions

Green composites composed of flax fibers (untreated, alkali treated and silane coupling treated) and biodegradable polyesters (PLA) were prepared by non-woven and hot-molding methods.

As a result of the activated soil test, lots of cavities are found on the surface of the composite caused by the biodegradation of the composite.

The addition of flax fibers into the composites causes acceleration of biodegradation for the preferential biodegradation of the flax fibers.

The biodegradability of the composites increases with the fiber content. The interface bonding property is in negative correlation with the biodegradability of the composite.

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A Study on the Antimicrobial Effect of Germitol on Some Pathogenic Microbes Observed on Building Worker's Clothes

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Abstract: The most clothing products are in direct contact to human body and they are a very suitable substrate for growing and increasing pathogenic microbes especially bacteria and fungus. In this research, the main aim is to investigate the antimicrobial effectiveness of germitol solutions finishing on Building Worker's Clothes with immersion method. 60 healthy male subjects (building workers) participated in this study. They were dressed in a cotton/polyester uniform for 14 days and some microbes found on them were investigated. The antimicrobial effect of different germitol solutions on the identified microbes was studied by the zone inhabitation method in vitro. In the next step the cotton/polyester uniforms were treated with different solutions of germitol like before and the antimicrobial effectiveness was assessed by colony count method at different times and the results were compared whit untreated ones. Some mechanical properties of treated cotton/polyester yarn were measured after 30 days and were compared with untreated one. Finally after finishing, scanning electron microscopy (SEM) was used to compare the surfaces of the finished and unfinished specimen. The results showed the presence of eight pathogenic microbes on building worker's clothes such as Escherichia coli, Staphylococcus aureus, Aspergillus and Mucor. The inhalation time for treatment on building worker's clothes improved. The amount of colony growth on treated clothes reduced considerably and moreover the mechanical tests results showed no significant deterioration effect of properties in comparison to the untreated yarn. The visual examination of the SEM indicated that the antimicrobial treatments were applied usefully on fabrics.

Keywords: Pathogenic microbes, Building Worker's Clothes, Germitol, Escherichia coli, Fusarium.

1. Introduction

A Wide array of microorganisms lives in a compost pile. Bacteria are especially abundant and are usually divided into several classes based upon the temperatures at which they grow best. The low temperature bacteria are the psychrophiles, which can grow at a temperature down to -10°C, but whose optimum temperature is 15°C (59°F) or lower. The mesophiles live at medium temperatures, 20-45°C (68-113°F), and include human pathogens. Thermophiles thrive at above 45°C (113°F), and some live at or even above the boiling point of water [17]. Most of the pathogenic microbes on textile goods are the mesophiles class [1].

The growth of microbes on textiles during use and storage negatively affects the wearer as well as the textile itself [1]. The growth of microorganisms on textiles inflicts a range of unwanted effects not only on the textile itself but also on the wearer. These effects

include the generation of unpleasant odor, stains and discoloration in the fabric, a reduction in fabric mechanical strength and an increased likelihood of contamination [2,18]. For these reasons, it is highly desirable that the growth of microbes on textiles be minimized during their use and storage [3].

In order to obtain the greatest benefit, an ideal antimicrobial treatment of textiles should satisfy a number of requirements [1,5]. Firstly, it should be effective against a broad spectrum of bacterial and fungal species, but at the same time exhibit low toxicity to consumers, e.g. not cause toxicity, allergy or irritation to the user. Antimicrobial-treated textiles have to meet standards in compatibility tests (cytotoxicity, irritation and sensitization) before marketing. Secondly, the finishing should be durable to laundering, dry cleaning and hot pressing. This is the greatest challenge as textile products are subjected to repeated washing during their life. Thirdly, the finishing should not negatively affect the quality (e.g.

physical strength and handle) or appearance of the textile. Finally, the finishing should preferably be compatible with textile chemical processes such as dyeing, be cost effective and not produce harmful substances to the manufacturer and the environment [2,11]. Several major classes of antimicrobial agents are used in the textile industry. They are generally not new as such and have been in use in other industries, e.g. as food preservatives, disinfectants, swimming pool sanitizers or in wound dressings. These agents are potent in their bactericidal activity, as indicated by their Minimal Inhibitory Concentration (MIC) values [2,9,10].

Quaternary ammonium compounds (QACs), particularly those containing chains of 12-18 carbon atoms, have been widely used as disinfectants [6,22]. These compounds carry a positive charge at the N atom in solution and inflict a variety of detrimental effects on microbes, including damage to cell membranes, denaturation of proteins and disruption of the cell structure [2,7,19]. During inactivation of bacterial cells, the quaternary ammonium group remains intact and retains its antimicrobial ability as long as the compound is attached to textiles [2,20].

Quaternary ammonium halide cationic surfactants are widely used for antibacterial surface-active and detergent properties [13-17].

Germitol shown in Fig. 1 is one of the conventional quaternary ammonium salts. Its solutions rapidly act as anti-infective agents with a moderately long duration of action. They are active against bacteria, some viruses, fungi and protozoa. Solutions are bacteriostatic or bactericidal according to their concentration [3,4,16].

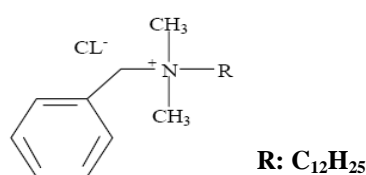


Figure 1 Molecular structure of Germitol.

The exact mechanism of bacterial action is unknown but it is thought to be due to enzyme inactivation. Activity generally increases with increasing temperature and pH. It has been used in textile industry, as an insecticidal or antimicrobial agent [6,7,19].

In this study a conventional antiseptic agent, Germitol was applied through immersing method for improving clothes' inhibition against some pathogenic microbes and the antibacterial effectiveness of the clothes was evaluated by standard test methods.

2. Material and Methods

2.1. Materials

Germitol was purchased from Asalib Co. (Table 1). The clothes were purchased from Kosha Co. Polyester/Cotton blend yarns were prepared from Kosha Co. (20/2 Nm). The pure bacteria were supplied by the Bouali Hospital, Tehran, Iran and all tests were done in the Laboratories of Tarbiat modarres University, Islamic Azad University Science and Research Campus Branch and Islamic Azad University of Shahre-Rey in 2009.

Table 1 Technical data of the applied Germitol

Trade name	Germitol 50
Product	A 50% v/v solution of alkyl benzyl dimethyl ammonium chloride, complying with BP 2003 and USP 26-NK 21 monograph.
Appearance at 20° C	Clear liquid
Color	Colorless to pale yellow
Density at 20° C	0.99 g.cm ⁻³
Viscosity at 20° C	120 CS
Assay (mmw = 349.8)	50 ± 1
Non-quaternized amine (mmw = 223.3)	0.5 %max
Sulphated ash	0.2 %max
pH (5% in water)	6.5-8.5

2.2. Methods

Immersing method was used for adding antibacterial finishes on the test clothes. A polyester/cotton blend fabric was used in this study because it is one of the most frequently used fabric for scrub suits, lab coats and uniforms [1]. Agar Diffusion Test is used in this research. The agar diffusion tests include AATCC 147-2004 (American Association of Textile Chemists and Colorists), JIS L 1902-2002 (Japanese Industrial Standards) and SN 195920-1992 (Swiss Norm). They are only qualitative, but are simple to perform and are most suitable when a large number of samples are to be screened for the presence of antimicrobial activity. In these tests, bacterial cells are inoculated on nutrient agar plates over which textile samples are laid for intimate contact. The plates are then incubated at 37 °C for 18–24 h and examined for growth of bacteria directly underneath the fabrics and immediately around the edges of the fabrics (zone of inhibition). No bacterial growth directly underneath the fabric sample indicates the presence of antimicrobial activity. The zone of inhibition should not be expected if the antimicrobial agent is firmly attached to the textile (e.g.

covalently) which prevents its diffusion into the agar. If the antimicrobial agent can diffuse into the agar, a zone of inhibition becomes apparent and its size provides some indication of the potency of the antimicrobial activity or the release rate of the active agent. Suspension Test is exemplified by AATCC 100-2004, JIS L 1902-2002 and SN 195924-1992. These methods provide quantitative values on the antimicrobial finishing, but are more time-consuming than agar diffusion tests. Typically, a small volume (e.g. 1 ml) of bacterial inoculum in a growth media is fully absorbed into fabric samples of appropriate size without leaving any free liquid. This ensures intimate contact between the fabric and the bacteria. After incubating the inoculated fabrics in sealed jars at 37°C or 27°C for up to 24 h, the bacteria in the fabric are eluted and the total number is determined by serial dilution and plating on nutrient agar plates. Antimicrobial activity, expressed as percentage of reduction, is calculated by comparing the size of the initial population with that following the incubation. Appropriate controls, e.g. samples that have gone through the same processing except the antimicrobial finishing, should be included in each experiment to ascertain that the observed decrease in bacterial number is truly due to the antimicrobial finishing. Choosing a calculation equation may be important. It has been observed that two different equations can produce very different results for the same set of data [2]. The test fabric, supplied by the Testfabrics Inc. with a code #7409, was 65% Dacron polyester/35% cotton.

60 Male healthy Building Workers (age 21 ± 3 years, stature 185 ± 3 cm, and weight 74 ± 10 kg) participated in this study. The subjects were dressed in a cotton/polyester uniform for 14 days. For investigating the kind of bacteria on clothes, (especially pathogenic ones), some fibres were cut from the clothes randomly and immersed in Thioglycolate and Nutrient broth mediums. After incubating for 24 hrs at 37 °C, the solutions of each media were sub-cultured in Nutrient and Blood agar mediums and after incubating in Nutrient and Blood agar mediums for 48 h at 37 °C the colonies of microbes were cultured by streak test method. For identifying the kind of cultured microbes the gram stain, catalase, oxidase, citrate agar, Christensen's urea broth and TSI agar tests were done. The cultured microbes were kept in skimmed milk as the next step. Ditch plates method was used for evaluating the antibacterial effectiveness of Germitol against the detected bacteria on the clothes. Ditch plates were prepared by allowing the Mueller Hinton Agar to solidify in a Petri dish and ditches (with

diameter of approximately 4 mm) were produced on it by removing the agar. Ditches were inoculated by different Germitol solutions (1/100, 1/500, 1/1000 and 1/2000 v/v solutions of Germitol). The dishes were incubated for 18 hrs at 37°C to let the Germitol solutions penetrate into the agar medium. Microbes (stored in skim milk) were mixed with a semi liquid Mueller Hinton Agar (Agar conc. <1%) and added to the inoculated plates. The plates were incubated at 37°C and the zone of inhibition at different time intervals (12, 24, 48, 72, 96, 120, 148, 172, 196, 220, 244 and 268 hrs) were determined. The positive results were repeated three times and the mean of the zone of inhibition was reported for 120hrs. fibers were sprayed with in different solutions of Germitol (1/100, 1/500, 1/1000, 1 /2000 v/v solutions of Germitol) and after drying they entered in plates containing the pure microbes and the zone of inhibition was observed until the zone of inhibition disappeared. Every 24 hrs the plates were replaced with new plates of pure Microbes. For comparing the antibacterial effectiveness of Germitol on clothes, the subjects were dressed in a new treated cotton/polyester uniform for 14 days and some microbes found on them were investigated again. First remained untreated and the second was treated with Germitol solution (1/500 v/v solution of Germitol by immersing method).

After using clothes used by workers, some fibers cut out of two samples and the previously described methods were used for culturing and separating the microbes and the antimicrobial effectiveness of Germitol on clothes was measured by colony count method.

Some mechanical properties of untreated and treated (1/500 % v/v solution of Germitol) polyester/cotton blend yarns were measured by Tensorapid (SDL Co.) after 30 days. The length of every sample was 300 mm and the speed of test was 999.9 mm/min. An International Standards Instrument ISX-430 SEM was used to compare the surfaces of the finished and unfinished specimen.

3. Results and Discussion

The presence of some microbes were proved in the experimental clothes, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, *Aspergillus*, *Trichophyton rubrum*, *Candida*, *Fusarium* and *Mucor*.

The antimicrobial effectiveness of Germitol solutions on the detected microbes after 120 hr is shown in (Table 2). According to the results the 1/2000

v/v solution of Germitol shows no significant antimicrobial effect.

Antibacterial effects of treated fibers with different solutions of Germitol assessed for the remaining time in the zone of inhibition are shown in (Table 3). Comparing results of the treated and untreated clothes is shown in (Table 4). It can be seen that the number of colonies growth were decreased about 56, 97 and 90% for *Staphylococcus*, *Pseudomonas* and *Escherichia coli* respectively.

Table 2 Antimicrobial effect of Germitol solutions on the found Microbes on clothes

The zone of inhibition (mm)of Germitol solution				
Microbe	1/100(v/v)	1/500(v/v)	1/1000 (v/v)	1/ 2000 (v/v)
<i>Escherichia coli</i>	17	14	12	*
<i>Staphylococcus</i>	15	13	*	*
<i>Pseudomonas</i>	13	13	*	*
<i>Trichophyton rubrum</i>	12	Less than 12	*	*
<i>Candida</i>	13	12	*	*
<i>Aspergillus</i>	14	12	*	*
<i>Fusarium</i>	13	12	*	*
<i>Mucor</i>	14	12	*	*

*: Showed no zone of inhibition

Table 3 Antimicrobial effect of treated clothes with Germitol

Maximum time of inhibition of treated fibers with Germitol solution (hr)				
Microbe	1/100(v/v)	1/500(v/v)	1/1000 (v/v)	1/ 2000 (v/v)
<i>Escherichia coli</i>	260	159	63	25
<i>Staphylococcus</i>	233	90	64	29
<i>Pseudomonas</i>	160	45	20	*
<i>Trichophyton rubrum</i>	40	22	*	*
<i>Candida</i>	241	72	*	*
<i>Aspergillus</i>	241	96	*	*
<i>Fusarium</i>	240	94	*	*
<i>Mucor</i>	217	72	*	*

*: Showed no inhibition time

Table 4 The Number of colonies growth on untreated and treated clothes with 1/100 Germitol (v/v) after 14 days

No. of colonies		
Bacteria	Untreated carpet	Treated carpet
<i>Staphylococcus</i>	8	6
<i>Pseudomonas</i>	19	1
<i>Escherichia coli</i>	21	1

The effect of 1/100 v/v solution of Germitol on some mechanical properties of treated clothes in comparison with untreated one is shown in (Table 5). There is no significant deterioration effect on the studied mechanical properties (e.g. the significant level of $\alpha = 0.05$, about Table 5). A successfully finished specimen should look smoother and more uniform compared to the unfinished specimen because the finish improves the surface properties of fibers and yarns. At 1,000 times magnification, the swatch with no treatment showed an unevenness on the fiber surfaces (see Fig. 2). Fiber surfaces with Germitol treatments looked smooth (see Fig. 3). The visual examination of the SEM indicated that the antibacterial treatments were applied successfully to second swatch.

Table 5 Effect of treating cotton/polyester yam with Germitol on some mechanical properties after 30 days

Mechanical property	Elongation at break (%)	Work of rupture (N.m)	Initial modulus (cN/Tex)	Tenacity (cN/Tex)
Untreated	1.6	934.5	98.5	760.3
CV	184.9	3200.1	58.6	120.5
Treated	985.5	0120.6	50.5	932.3
CV	020.9	1400.9	02.3	126.2



Figure 2 Scanning electron microscopy no treatment swatch.



Figure 3 Scanning electron microscopy of Germitol treated swatch.

4. Conclusions

Germitol was chosen for this study because it is a common antiseptic and it belongs to the Group of cationic surface active agents. Considering its charge it can act link a cationic dye and tend to take up and hold on the surface of natural substrate such as polyester/cotton blend. According to the results the presence of some pathogenic microbes on the clothes including *Escherichia coli* and *Staphylococcus* which can be causing many infections was confirmed. So it is worthy to enhance the antimicrobial activity of the clothes with a proper antimicrobial finishing. Although the kind of microbes on the clothes depends considerably to the environment of course, but it was shown that treating fabric with Germitol inhibits considerably the growth amount of studied bacteria and in some cases up to 99%.

The wash fastness or durability of the effect against washing of the treated clothes in the study was not under attention because the interval of washing periods for militarism textile clothes are not short and during these intervals usually the activity of the antibacterial agent vanishes, as it was seen in the case of Germitol whose maximum inhibition time with a high concentration (1/100 v/v) was just 260 hrs. Fiber surfaces with Germitol treatments looked smooth and the antibacterial treatments were applied successfully to improve surface of the clothes.

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Quantum Cellular Automata as Bio-information Processing Tool--A Perspective

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Abstract: It was proposed that Quantum Cellular Automata concept could be used as a biological information processing tool in a broad sense to perform nano-bio simulations using QCA concepts. Molecular Quantum Cellular Automata (QCA) is an exploratory computing tool in which information is encoded in the electronic charge configuration of a QCA cell (built from one or two individual molecules). The charge interaction between neighboring cells enables the transmission and processing of information. It is understood that the biological systems work in a complex matrix of electrical responses to control various activities based on a number bio-physical publications. Biological Systems are open, continually exchanging matter and energy with their surroundings. QCA plays an important role not only because it provides a solution to build circuits at nano-scale, but also offers a new methodology of computation and information transformation. In this present work simulations of basic biological concepts of Proto-cell were performed based on QCA theory using open source QCA simulation software, provided an analysis on how to model and simulate cellular level based mathematical concepts using logical elements. QCA circuits are extremely area efficient for digital circuits design. As the area of one QCA cell is 100 nm^2 with 10 nm cell dimensions, the total area of a QCA full-adder is around 0.04 mm^2 . The first step is to construct molecular computational systems to be able to develop simple units. The possibility of making logic gates with Proto-cells implies that we are not very far in reaching this objective. NOT, AND and XOR gates have been constructed with Proto-cell. The inputs and the output of these gates have the same nature this is why it is quite reasonable to think about the interconnection of these basic elements for various applications.

It is certain that in the near future quantum cellular automata could form an excellent simulating platform for Bio-Sensor Research. In-addition to the above mentioned observations these computational aspects could be extended to design and implement intelligent-textiles for Biomedical and Space Applications.

Keywords: QCA; proto-cells; molecular computation; bio-sensors; intelligent-textiles

1. Introduction:

In 1959, Richard Feynman, a future Nobel Laureate, gave a visionary talk entitled. "There's Plenty of Room at the Bottom" on miniaturization to nanometer-scales. Later, the work of Drexler [4] also gave futuristic visions of nano technology. Feynman and Drexler's visions inspired many researchers in physics, material science, chemistry, biology and engineering to become nano technologists.

Cells are the building blocks of biological complexity. They are complex systems sustained by the coordinated cooperative dynamics of several biochemical networks. Their replication, adaptation and computational features emerge as a consequence of appropriate molecular feedbacks that somehow define what life is.

Even though the scientific community has reached a consensus on the requirements and properties of a

minimal living system (Pohorille & New 2001; Deamer 2005), the materialization of this vision into a concrete laboratory prototype is still incomplete. In this theoretical review we further explore and highlight the concepts and rationale behind the design, simulation and construction of artificial cellular systems and usage of the same for the Bio-Sensoric applications in diverse areas of Nano Technology domain.

To achieve the above mentioned aspects we observe proto-cellular activities as computational logic gates, including protein-directed genetic information that led to a more modern DNA-based information system, are the link between chemical and biochemical unity of proto-life and modern life, that this wholeness is part of nature encompassed by cosmo-genesis, and that the mind of man can comprehend this unity.

2. Descriptions

2.1 Basic molecular and cellular signaling mechanisms

The fields of biological, medical physics and biomedical engineering are broad, multidisciplinary and dynamic, they lie at the cross roads of frontier research in Physics, biology, chemistry and Medicine, established in the emerging areas of Science including molecular membrane/mathematical Bio Physics, Photo synthetic energy harvesting and conversion, information processing, physical principles of genetics, sensory communications, automata networks, neural networks, cellular automata and Quantum Cellular Automata.

Cells partition their core cellular processes into a fixed infrastructure and a control layer. Proteins in the control layer function as signals as receptors of the signals, as transcription factors that turn genes on and off as signaling transducers and intermediaries. The Signaling and regulatory proteins and associated small molecules make contact with the fixed infrastructure responsible for metabolism, growth, replication and reproduction at well defined control points where the signals are converted into cellular responses.

Biological systems are stunningly well engineered. Proof of this can be seen all around us. The Good engineering of biological systems is exemplified by the above mentioned partition of cellular processes into the fixed infrastructure and the control layer. This makes possible machinery/Bio-devices that always work the same way in any cell at any time and whose interactions can be exactly known while allowing for the machinery's regulation by the variable control layer at well defined control points.

Another example of good engineering design is that of modularity of design. Proteins especially signaling proteins are modular in design and their components can be transferred, arranged and rearranged to make many different proteins. The proteins components interact with one another in the largely independent components, but also in the DNA regulatory sequences. These sequences serve as control points for the networks that regulate gene-expression.

One of the great conceptual breakthroughs in investigating the control mechanisms of layers is that the signaling proteins involved in Cell-Cell communications are organized into signaling pathways. In a signaling pathway, there is a starting point, usually a receptor at the plasma membrane and an ending point (control point), more often than not a transcription regulatory site in the nucleus and there is a linear route leading from one to the other. In spite of the enormous

complexity of the metazoans, there are only about dozen or such path ways.

For Example, some pathways are prominent during development and are best understood in that context. Other path ways are associated with stress responses and are best understood within that framework and still others are associated with immune responses.

Signaling and the cellular responses are controlled by a plethora of positive and negative feedback loops. The presence of the feedback complicates the simple picture of a linear path way, but it is essential part of the signal ling process. Positive feedback ensures that once the appropriate thresholds are passed there will be a firm commitment to a specific action and the systems will not jump back and forth between alternative responses. Negative feedback generates the thresholds that ensure random excursions and perturbations do not unnecessarily commit the cell to some irreversible response when it ought not to and it permits the cells to turn off the signaling once it has served its purpose. Hence to model fully functional and highly efficient biosensors understanding of linear signaling path ways is important.

We further point out that this particular topic is a vast one and it is not fully possible to discuss all the related concepts on an in-depth basis in just one review.

Proto-cells as computational logic gates that were considered for modeling based on QCA.

2.2 Enzyme-driven, information-free proto-cells

Based on an early suggestion by the Russian bio-mathematician Nicolas Rashevsky, it has been shown that the stability of a spherical closed membrane can be lost by providing an appropriate set of metabolic reactions (Rashevsky 1960). Specifically, Rashevsky conjectured that a vesicle having enzymes allowing metabolic reactions occur inside the compartment could experience a destabilization eventually leading to cell division. We illustrate the generalization using Figure 1a and explain further using Figure 1b & Figure 1c as basis for our QCA simulation

It has been recently shown that this is the case for a very simple model involving, for simplicity, two enzyme molecules, placed at the two opposite poles of the cell (Figure 1b). If these enzymes catalyze a reaction transforming a given precursor (R in Figure 1b) into a new molecule G, then close to the location of each enzyme, the concentration of G would increase and eventually trigger a heterogeneous pressure

distribution along the membrane (Maci'a & Sole' 2007a). Additionally, surrounding lipid molecules L become incorporated into the vesicle as membrane bounded molecules (here indicated as L_m). Since the process is necessarily linked to enzymatic activity, the splitting is a single event, not able to be repeated.

2.3 Turing-like proto-cells

These model proto-cells introduce a very simple coupling between a reaction–diffusion (RD) system defining a metabolism and membrane dynamics (Figure 1b). In this scenario, there is no need for spatially localized enzymes. Instead, externally provided precursors R1, R2 are supplied, entering the membrane and being transformed into new molecules through a set of simple reactions. The reactions inside and outside the cell are represented by a set of n RD equations (Murray 1989), namely

$$\frac{dC_i}{dt} = \Phi_i(C_1, \dots, C_n) + D_i \nabla^2 C_i,$$

with $i=1, n$, the index associated to the i^{th} morphogen having a local concentration C_i . Each term Φ_i describes how the i^{th} molecular species reacts to the other molecules. The last term on the right hand side is the diffusion term accounting for the spontaneous, random movement of molecules through space. However, the formalism needs to be extended by incorporating a changing boundary which now acts as a permeable membrane, also coupled to the reactions described by Φ_i . These reactions will define the proto-cell metabolism.

Further information could be found in Maci'a & Sole' 2007b. & [5].

2.4 Diagrams of proto-cells based on the models mentioned above

Figure 2a Proto-cells could be used to perform simple types of computations. Here, two very simple examples are given, for illustrative purposes only. Computation is linked to cell division (D): as the output to some given inputs, the cell can ($D=1$) or cannot ($D=0$) replicate itself. Here, we use a proto-cell model (Maci'a & Sole' 2007a) where two enzyme molecules (E) are used as catalytic systems, transforming an external precursor R into a new molecule G. If the enzymes can be repressed by using

given inhibitor molecules (I), then simple switches can be made. In(a,b), we show how to implement a NOT gate (c), whereas the second example (d, e) illustrates a more complex, two-input gate (f) (the NOR gate).

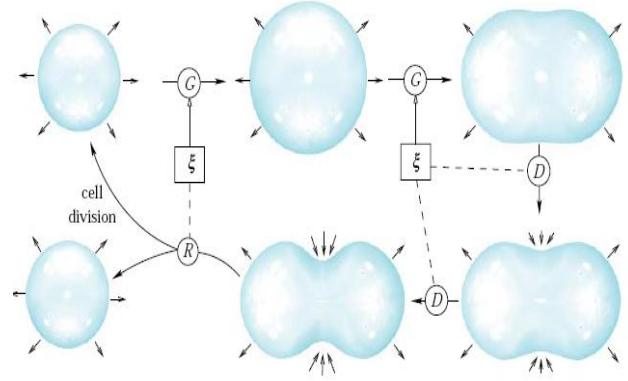


Figure 1a A schematic self reproduction-cycle of protocell, requiring growth(G), deformation(D) and replication(R) phases in order to be completed. When dealing with nanoscale phenomenon both internal and external fluctuations(ξ) are also considered. Ref [5].

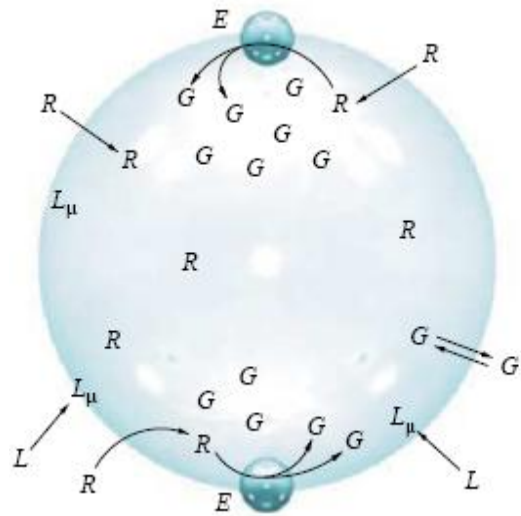


Figure 1b Simple systems with a simple replication cycle. Ref [5].

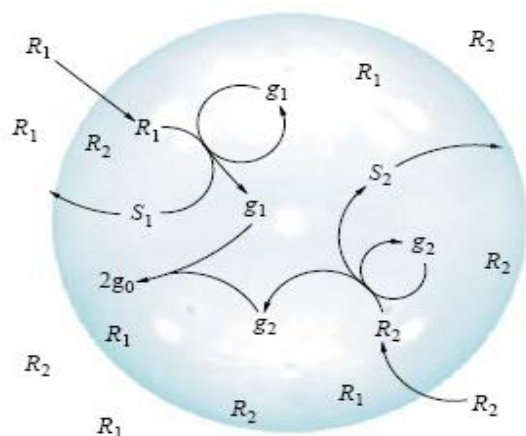


Figure 1c Turing proto-cells. Ref [5].

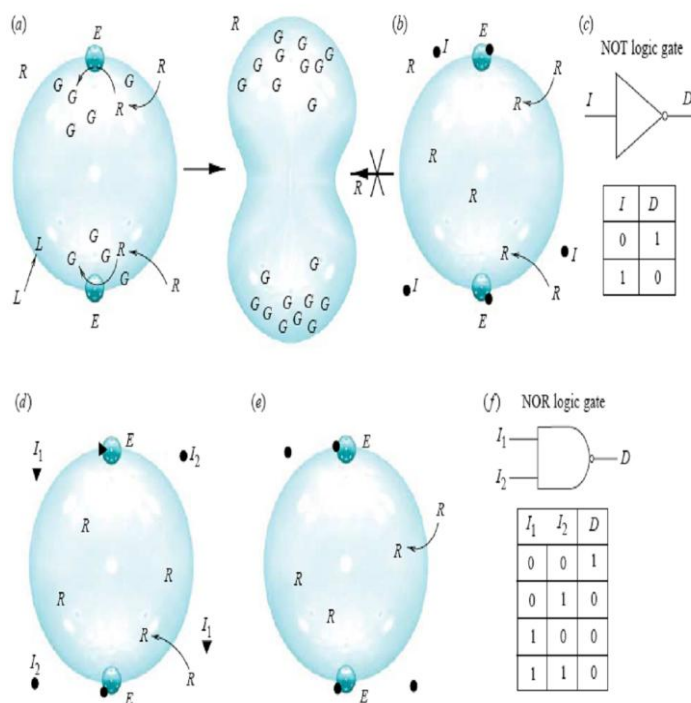


Figure 2a Logical Gates. Ref [5].

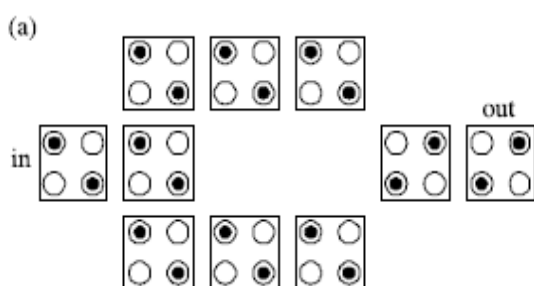


Figure 3a Simple NOT GATE implementation using QCAD Simulation Tool. In a similar way NOR GATE

implementation could be realized to study the cellular switching mechanism in Complex Systems. [NOT Gate depicted in Fig2a is implemented.]

2.5 QCA application analysis and discussions

Most of these basic cell models can be used to implement computational tasks. Here, computation means some sort of predictable response to external signals. In Figure 2a, we show two simple examples of explicit designs of two basic logic gates (the NOT and NOR gates, respectively) from the first model described in this section.

Here, the external signals correspond to some type of inhibitor of enzyme activity.

As a measure of the output, we use cell division: the output D will be one if division takes place and zero otherwise. In other possible scenarios, the output can correspond to some type of produced molecule resulting from cell reactions.

The NOT gate can be obtained by using an enzyme having a single inhibitor I: under the presence of I no division occurs, whereas if absent the vesicle experiences reproduction. A simple generalization of this using two enzyme inhibitors (here indicated as I1 and I2) leads to a NOR gate: unless both are absent, no reproduction can occur. Although these are rather trivial examples, they illustrate possible ways of designing simple types of computational cell structures. Once we incorporate as inputs the produced molecules or alternatively introduce different types of cells responding to different signals, it is not difficult to generate (at least at this theoretical level) more complex systems able to describe switches or even memory structures. It is worth mentioning that very complex computational devices (including Turing machines) are currently being developed experimentally at the molecular level (Shapiro & Benenson 2006). These molecular automata could benefit in the future from the current advances in Nano-Bio Computational platforms and devices.

3. Conclusions for future work

In this review we have considered simple biological concepts, with the help of QCA concepts and available QCA simulation software provided an analysis on how to model and simulate cellular level based mathematical concepts using logical elements. It is quite challenging to model bio-nano logical circuits and implementation of digital devices, it is hoped that this review could form a basis or useful reference in

the vast field of computational molecular systems. Information Technology forms the Key to the Chemical Artificial cell based bio-medical devices and in the evolving field of Intelligent Textiles

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Advances on the Clinical Application of Stent Placement for Colorectal Cancers (CRCs)

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Abstract: Colorectal stents have been reported as an effective alternative to surgery for the palliation or as a “bridge to surgery” for patients with obstructing colorectal cancers. This review discusses the need for colorectal stent placement, distinction from colostomy and resection with primary anastomosis, the requirements for placement, and the many efforts over the past century to accomplish this goal experimentally and clinically. This work briefly presents both commercially available stents and relevant newly developed experimental bio-functional materials, including elastic polymers (polyurethane), the biodegradable and bioresorbable materials, and the naturally occurring materials, focusing on their potential applications in the development of future colorectal substitutes. This paper also examines the critical issues and scientific challenges that require further research and cooperation of multidisciplinary teams.

Keywords: colorectal stents; colorectal cancer; obstruction; palliation; biomaterials; multidisciplinary.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers in terms of both incidence and mortality in the world [1]. According to the World Health Organization, globally there are more than 800,000 newly diagnosed cases of colorectal cancer each year, with an overall annual mortality of more than 500,000 [2-4]. In particular, over 30,000 new cases are diagnosed in England and Wales each year, of which about half will have colorectal cancer registered as the underlying cause of death [5,6]; in the United States in 2009, 146,970 new cases of CRC were diagnosed with 49,920 dying of disease [7]; it comprises 10% of the over 500,000 annual cancer deaths, making it the third most common cancer in men and women; in the UK, it is about 25,000 new cases annually [3,8,9]. The same tendency is in China mainland and HK area and other countries and areas [10-12]. These data strongly suggest that more attention should be paid to the prevention and control of CRCs.

CRC is the principal cause of large bowel obstruction. Up to 75% of colorectal cancers occur in the left colon and a significant proportion (8-29%) of these will cause acute large bowel obstruction [12-14]. In 10-20% of all cases, partial colonic obstruction will develop, and complete obstruction occurs in an additional 5-20% [15,16].

Although surgery is considered the standard treatment for neoplastic large bowel obstruction, complications after surgery for large bowel obstruction are relatively frequent, with reported mortality rates ranging from 8.8% to 27% [17-21]. In addition, many of these patients couldn't undergo curative surgical treatment because they were unfit for general anesthesia, in poor general health, nor had concomitant disseminated neoplastic disease [22]. However, in case of without surgery, these patients have a median survival of 7 months [23]. Death occurs within 5 years from diagnosis in 80-90% of cases [24]. Moreover, these patients have complications that considerably worsen their quality of life [25], including intractable pain, occlusion, bleeding, perforation, and septic diseases [26]. Therefore, only palliative treatment is possible relief for these symptoms.

Resection can provide excellent palliative treatment, but this treatment has a morbidity rate of 4%-60% and a mortality rate of 3%-11% [8]. Colostomy to resolve bowel decompression is an alternative treatment, but one with high morbidity and mortality rates in patients with large-bowel obstruction caused by neoplasm [22]. Thus, resection and colostomy are also avoided, and space is created for other types of complementary therapeutic symptom relief.

2. Stent placement

A recent systematic review found colonic stenting, when used as a bridge to surgery, can prevent acute colonic obstruction in patients who have impending obstruction detected either by colonoscopy or by diagnostic imaging (computed tomography scanning, barium enema or water-soluble enema). This method is to be safe, with low mortality rates, and to have technical and clinical success in 92% and 88% of cases, respectively [27]. Earlier studies [28-30] have also demonstrated colonic stenting under combined endoscopic and fluoroscopic guidance in acute colonic obstruction is a minimally invasive, low-risk treatment for rapid relief of acute ileus. It allows relief of obstruction while avoiding stoma formation in palliative cases, or it facilitates the completion of staging investigations, bowel decompression and preparation as a “bridge to surgery” for those with resectable disease [31]. It allows optimal preoperative planning and gives the opportunity for the administration of any neoadjuvant chemo/radiotherapy if required [32]. In addition, it can provide time for systematic support and obviate the need for faecal diversion or on table lavage. The method rapidly resolve bowel obstruction and/or bleeding without the risk involved in surgical treatment [33].

2.1 Basic principles

Stents are composed of a variety of metal alloys or compound materials with varying shapes and sizes depending on the individual manufacturer and organ of placement, which will allow bowel motions to pass through an area of the bowel which has become

blocked, either by scarring or by tumor [34]. During patient’s colonoscopy, the blockage will be passed with the endoscope. This may require the blockage to be stretched (dilated), using a balloon passed through the endoscope. A thin wire will then be placed through the blockage and the endoscope removed. The stent will then be passed over the wire, through the blockage using x-ray control. At this point the stent is “released” which allows it to open up and hold open the blockage.

2.2 Materials

Since 1992 an increasing number of reports have described the use of stents for recanalization of malignant and benign bowel obstruction [35-40]. The common materials approved by FDA are listed in Table 1, 2 as below [41-48]. As the development of molecular biology and biology engineering in recent years, some new bio-functional materials such as fibrin glue have been used for the treatment of colorectal diseases [25].

2.3 The types of stents

The first SEMS, the Z stent (Wilson Cook) was designed by Gianturco for use in vascular stenoses and described in use by Wright et al. [48] in 1985. They are available in a variety of diameters and lengths and are found in helical (eg, Esophacoil, Instent, Eden Prairie, MN), knitted (eg, Ultraflex, Boston Scientific, Oakland, NJ) and braided (Wallstent, Schneider, Zurich, Switzerland) varieties [31,49]. Different types of colorectal stents are as shown in Figure 1.

Table1 FDA-approved expandable metal esophageal stents

	Ultraflex	ZStent
Material	Nickel Titanium	Stainless Steel
Delivery system diameter (F)	16	28
Covering	Yes	
Degree of shortening	30-40%	0-10%
Radial force	+	++
Design	Mesh	zig-zag
Lumen diameter flanges	23, 28	21, 25
Lumen diameter shaft	18, 23	18

Table2 FDA-approved self-expanding metal stents

Type	Metal and Design	Delivery System (F)	Deployed Length (cm)	Deployed Shaft Diameter (mm)	Deployed Flare Diameter (mm)
Enteral Wallstent	Stainless steel, wire mesh	10 Fr	6,9	18-22	N/A
Z stent	Stainless steel open wire spring	30Fr	4,6,8,10,12	25	35

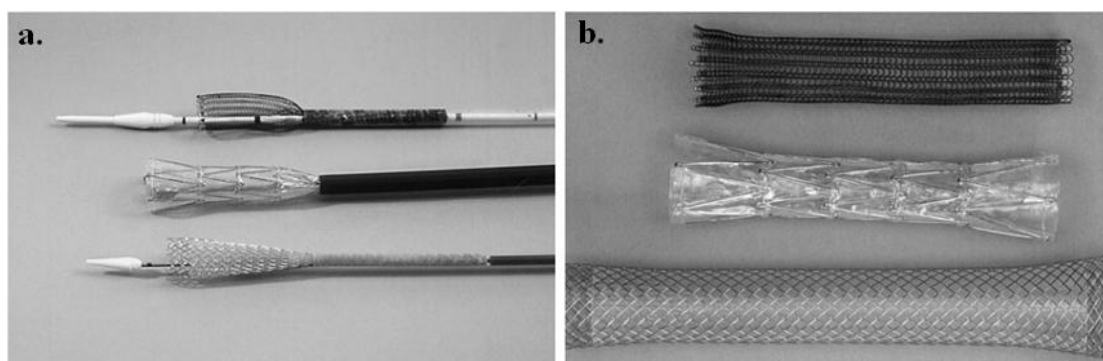


Figure 1 Photographs of colorectal stents.
(a) Depolyment systems. (b) Deployed covered and uncovered stents.

2.3.1 Uncovered stent

Kim et al. [50] explored metal stents that lack a covering membrane (uncovered stents), with good flexibility and stability and therefore easier to deploy [51-60], which have been successfully used for both preoperative decompression of acute neoplastic large bowel obstruction and long-term palliation of patients who are not candidates for surgery [49,50,61-63]. However, stent occlusion due to tumor ingrowth has been reported in 10% to 30% of cases in which uncovered stents were used [62,64].

2.3.2 Covered stent

Repici et al. [62], Tominaga et al. [65] reported the primary advantage of stents with covering membranes is the prevention of tissue ingrowth (tumor or mucosal hyperplasia, and subsequent recurrent obstruction) through the mesh wall for patients who require stents for long-term colonic decompression [66,67]. This advantage is often outweighed by the stent's increased rigidity and tendency to migrate. Guan et al. [68] investigated the stent membranes using 5-FU and taxol particles; once deployed, the stent material becomes incorporated into both the tumor and surrounding tissue by pressure necrosis. This reaction allows both on curing colorectal tumors directly and anchoring of the stent and helps to prevent stent migration. With the use of covered stents, this integration does not always occur and a higher rate of stent migration is seen. Stent embedment accounts for some of the adverse effects seen when the pressure from expansion causes stent material to erode [64, 69].

2.3.3 Self-expanding metallic stent (SEMS)

Self-expanding metal stents (SEMS) are currently

used to treat variable sites of the gastrointestinal tract and biliary obstruction [70-72]. Three different self-expandable colonic stents are approved by the food and drug administration (FDA) in the United States for treatment of malignant obstruction [37,73,74]. These are (a) the colonic Z-stent (Wilson–Cook Medical, Winston-Salem, NC) with diameters of 28 mm flanged ends and 25 mm mid-body; (b) the Enteral Wallstent (Microvasive Corp., Natick, MA) with dimensions of 20 and 22 mm diameters; and (c) the Ultraflex Precision Colonic Stent (Microvasive, Boston Scientific Corp., Natick, MA) with a 30 mm diameter proximal flare and 25mm body [75].

The advantage of using the Enteral Wallstent over the other colonic stents is the much longer and smaller diameter (10 Fr) delivery system that allows passage of stents directly through the working channel of the endoscope. A theoretical advantage of the Wilson-Cook Z-stent and the Ultraflex Precision stent is the larger diameter of the lumen compared to the Enteral Wallstent. One further advantage of the Z-stent is that it does not shorten during deployment. [75]

2.3.4 Biodegradable stents

In 1996, Goldin et al. [76] firstly reported a kind of biodegradable stent. Sandha et al. [77] indicated this kind of novel stent is unnecessary to be recollected and the ideal colorectal stent. Guan et al. [68] indicated this biodegradable stent can be absorbed and catabolized in alternative term, which facilitates both local medicine release and temporary bracing; it can provide time for systematic support and obviate the need for faecal diversion or on-table lavage [78].

2.3.5 Other stents

Guan et al. [68] reported the radioactivity stent binding

with ¹⁹²Bo, Strontium and ¹²⁵Iodine particles etc curing tumors directly; genophore-membrane stent was used to change the genetics codes of the surrounding tissues by medical intervention.

2.4 Stent selection

Baron et al. [74] indicated the stent selection is subject to many factors, including the tumor sites, size, potential passages, fistula, degree of obstruction, length of stricture and personal experiences. Guan et al. [68] thought the uncovered and covered stents are often used for large bowl obstruction as a role of bridge to surgery and the long-term colonic decompression, respectively.

2.5 Description of procedure

Stent placement can be divided into several distinct phases and is usually accomplished with a combination of endoscopic and fluoroscopic techniques [37,79-82].The Schematic of non-through the scope (TTS) stent placement is briefly presented as 5 steps: (a) A guide wire has been passes across the lesion under endoscopic and fluoroscopic guidance and exchanged for a super-stiff wire. (b) The endoscope is withdrawn leaving the guide wire in place across the stricture. (c) The predeployed stent is passed over the guide wire under fluoroscopic guidance. (d) The endoscope is reinserted alongside the predeployed stent to confirm proper positioning across the lesion before and during deployment. (e) Under endoscopic and fluoroscopic guidance, the stent is deployed. The stent application procedure is as shown in figure 1 [83-87].

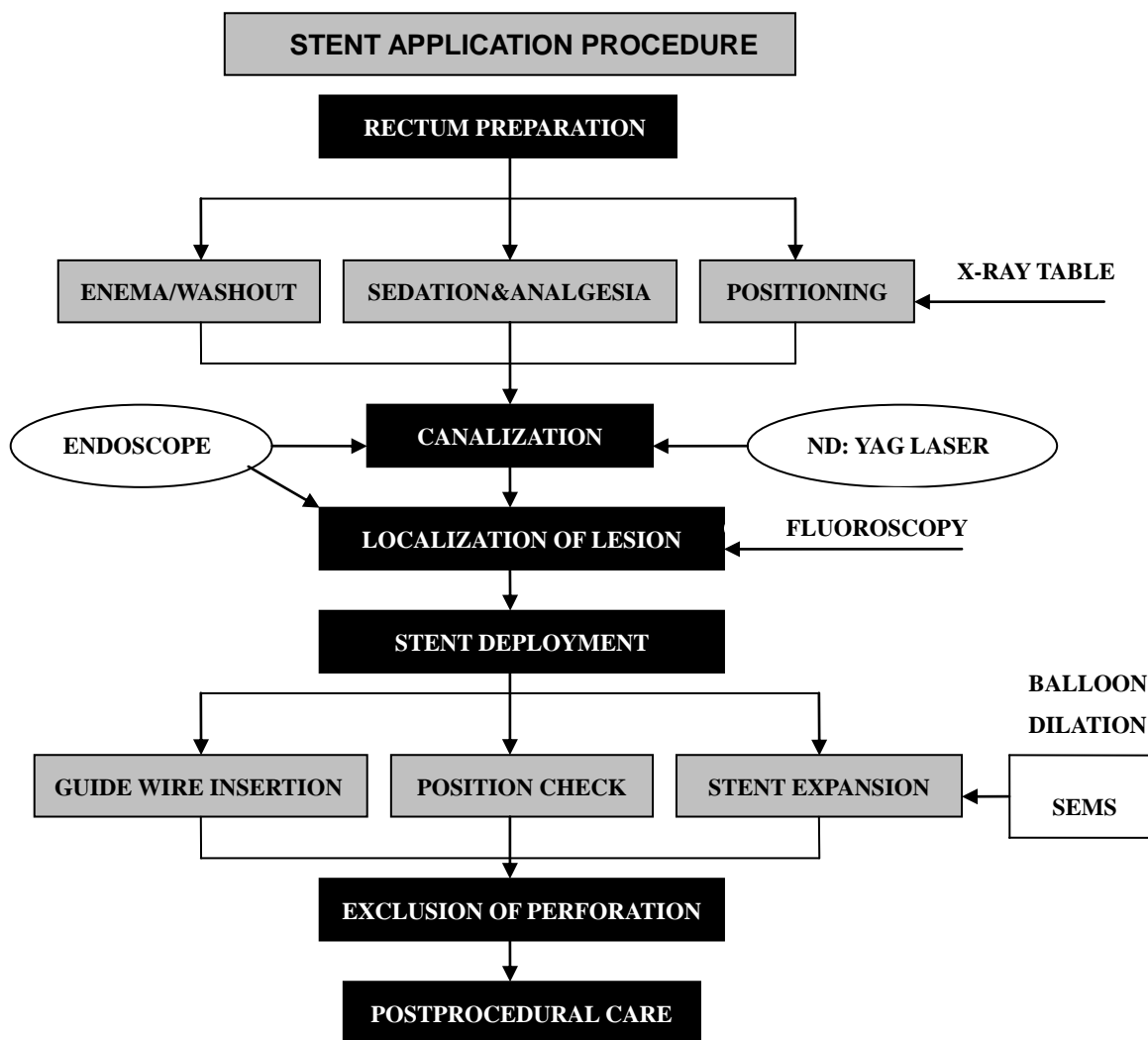


Figure 1 Stent application procedure in patients with large bowel obstruction.

2.6 Indication

Many different endoluminal stents have been used by various investigators for different indications.

2.6.1 Malignant Fistulae

Patients with malignancy within the pelvis may suffer from fistulae to surrounding structures such as the vagina or bladder. Anastomotic leakage is the common and severe complication after colorectal surgeries. Intestinal canal being drawn repeatedly, over-cutting of the colorectal membrane all lead to the over tension at the stoma, which causes the phenomena of anastomotic leakage. So the key of surgery is to allow free drainage for the healing of leakage. Colorectal stents have been used to close such fistulae and allow for nonsurgical palliation [34,88-91].

2.6.2 Obstruction

It has been estimated that between 8% and 29% of patients with colorectal cancer will have complete or partial intestinal obstruction [15,92,93]. Colorectal stent placement may serve as a palliative modality of obstruction.

2.6.2.1 Benign stricture

Benign stoma stricture is the common complication, especially at the stoma of low rectum [94]. Guan et al. [95] indicated that patients with local pelvic tumors (ovarian carcinoma) or metastatic disease to the pelvis and colonic obstruction may achieve palliation of obstruction with colonic stenting. Forshaw et al. [96] reported SEMS was used for curing benign stoma stricture. However, this work has not given sufficient weight to the practical use in clinic [94,97]. Meisner et al. [98] thought SEMS placement didn't induce good result but with a high complication. There is few of materials at present mentioned the SEMS application status curing benign stoma stricture or obstruction.

2.6.2.2 Malignant obstruction

Gukovsky-Reicher et al. [99] concluded that it's difficult to find effective cure solutions to malignant diseases. Carter et al. [100] and Ren et al. [101] reported that stenting technology is successful both in

curing primary colorectal cancers and large bowel obstruction caused by benign diseases. Shang et al. [102] illustrated that stent placement is a perspective solution for CRC, avoiding colostomy induced high morbidity and mortality.

Mauro et al. [103] reviewed the indications of stent placement as follows, (a) temporary decompression; It allows optimal preoperative planning and gives the opportunity for the administration of any neoadjuvant chemo/radiotherapy if required. (b) palliative treatment; it can provide palliation of obstruction in patients who are either unsuitable for laparotomy or in capable of surviving [2,104-105]. Evidence suggests that colorectal stents offer good palliation, and are safe and effective as a "bridge to surgery". Stent usage can avoid the need for a stoma, and is associated with low rates of morbidity and mortality [101,104-108].

2.7 Complication

Stent placement may be resulting in varying rates of success and complications, some of them are fatal cases including stent reobstruction, stent migration, bleeding, perforation, fistula and so on [109-118]. Suzuki et al. [119] analyzed the complications (Table 3, 4) were defined as early and late (occurring ≤ 30 days or >30 days after the procedure, respectively). It was shown that in table 3 the complications of early 30 days are more than in late days. Khot and colleagues [27] were able to identify the complication in 598 instances. From table 4, it was analyzed that the migration is the most among all complications. All of reports indicated stent placement is a feasible, effective and alternative to surgery for acute colorectal obstruction [120].

Carter et al. [100] concluded that two important tips are helpful to avoid intra-procedural perforation. The first is limiting the amount of air insufflation during the exam, especially in patients with a dilated cecum. The second is avoiding present or post-stent dilation. Baron et al. [74] analyzed the improper stents cause reobstruction of large bowel. Keymling et al. [121] and Lagattolla et al. [122] explored the restenting is the most effective method, namely, "stent-graft". Few report presents bleeding occurrence severely [123,124]. Spinelli et al. [125] and Mauro et al. [103] indicated perforation is a fatal complication and analyzed the reasons causing perforation. If balloon dilatation was avoided, the perforation ratio would be below 5%.

Table 3 Complications after stent insertion

Nature of Stricture	Early(≤30 Days)		Late (>30 Days)	
	Complication	n	Complication	n
Benign (n=6)			Migration (2 and 4.5 mo)	2
			Reobstruction (24 mo)	1
Malignant (n=30)			Fistula (eroded into terminal ileum) (3 mo)	1
	Migration	5	Reobstruction (6, 11, and 14 mo)	3
	Perforation	2	Fracture (5 mo)	1
	Incontinence	1		
	Bleeding (self-limited)	3		
	Inflammation/ulceration	2		
	Reobstruction	1		
Total (n=36)		14		8

Remark: 42 insertions in 30 malignant and 6 benign strictures.

Table 4 Complications of colorectal stent insertion [27]

Complication	number	Mean incidence
Death	3	1%
Bleeding	27	5%
Perforation	22	4%
Migration	54	10%
Reobstruction	52	10%

Total instances: 598

2.8 Efficacy / Safety

Colorectal stents have been used to relieve acute obstruction of the lower bowel for the last ten years [6,126-128]. Evidence on which to base estimates of their efficacy and safety remains sparse. They have not been the object of any randomized controlled trials, though numerous case reports and small case series have established that by their use obstruction can be successfully relieved.

In a literature search by Khot and colleagues [27] provided satisfactory evidence that stent placement is a relatively safe and effective alternative to colostomy, either as a “bridge to surgery” or for the palliative treatment of inoperable malignant disease.

Another reviewer Tack et al. [39] who included many of the same reports, reported the length of follow-up [129,130]. Diaz et al [131] followed up 16 consecutive patients after palliative stent placement until death, or termination of the study at 44 months. The stents successfully resolved the clinical obstruction in all patients except one who required colostomy. No patients showed clinical symptoms of obstruction at the time of death or termination of the study. Camunez et al. [105] used stent placement as palliative treatment in 35 patients with a mean follow-up of 93 days. It was proved that it's safe and effective.

We may conclude from the above that for present purposes palliative stent use may offer improved quality of life after placement, depending on the stage of disease, and is a satisfactory alternative to colostomy as a bridge to surgery.

2.9 New comprehensive stenting approach

Over the last 7 years, CRC death rates in men and women have decreased steadily (17% and 24%, respectively). These advances may be attributed to a better understanding of the disease genetics, improved surveillance, technical advances in the operations, increasing indications for the use neoadjuvant and adjuvant chemotherapy, radiation and better palliation [7].

2.9.1 Stenting integrated with laser technology

Nowadays, comprehensive stent treatment techniques are becoming more reliable. They are the combination with laser (Nd: YAG) photocoagulation, electrocoagulation, cryotherapy, and the placement of metal stents in patients with a limited life expectancy. These methods rapidly resolve bowel obstruction and/or bleeding without the risk involved in surgical treatment [17,33].

The advantages of endoscopic placement of stents over interventional radiologic placement occurs because of greater accessibility to these sites and improved mechanical advantage of being able to pass some stents directly through the working channel of the endoscope.

2.9.2 Stenting integrated with chemotherapy and radiation

Many of these patients cannot undergo curative surgical treatment because who are unfit for general anesthesia, are in poor general health, or have concomitant disseminated neoplastic disease. Stent placement integrated with chemotherapy and radiation is the better solution for increasing survival by 3-6 months without increasing adverse effects on quality of life [13,16,117,118]. In the work of Camunez et al. [105] and Shang et al. [27], they presented that external radiotherapy used alone eases pain in a high proportion of patients with locally advanced rectal cancer. In some patients, tumors have gone into complete remission or regressed sufficiently to permit curative surgery after prolonged fractionated radiotherapy of 45-50 Gy.

3. Future studies and directions

Despite the recent advances in stent technology in which it is still facing many problems, the search for the ideal enteral stent continues. The current studies mainly focus on the clinical therapies. Lan et al. [25] indicated the importance of using biomaterials for colorectal stents. However, few works are found to optimize the design and fabrication of new stents using bio-functional materials.

3.1 Biomaterials innovation

Biomedical materials and their production have been developed rapidly in the recent years. The global consumption of biomedical fibers and their products in 2006 reached above 318,000 tons; the current annual growth rate is about 3.5%. The growth rate of biomedical textiles has reached 25%-30% (data of 2008).

Synthetic polymers, used extensively, can be divided into permanent e.g. polyamide, polyester, polyethylene, polypropylene, PTFE and polyurethane and biodegradable which are mainly used in sutures and tissue engineering structures e.g. PHB polyhydroxybutyrate, PCL polycaprolactone, PLA poly(lactic acid), PMA polymerthylacrylate, PGLA copolymer of PLA and PGA poly(glycolic acid) and PLLA poly(L-lactic acid) [136-148]. Natural biological fibers include chitin (from the cells of crustacea) a polysaccharide renowned for its wound healing properties and incorporated into wound dressings; collagen (a fibrous protein found in connective tissue, tendons, etc.) used in cell engineering structures, for example artificial skin; and alginate fibers which can interact with the wound to form an absorbent gel, that

acts as a protective barrier and still allows the wound to breathe [149-167]. However, the colorectal stents using the above biomaterials are sparse.

3.2 Design and fabrication innovations

On a worldwide basis, biomedical textile stents with braiding, knitting and woven technologies have been in routine clinical use for nearly five decades and have been implanted in hundreds of thousands of patients, due to their many advantages, such as good compatibility, softness, easy-bending, small quantity and a low-strength request on yarns [168-172]. Examples of these products are stents/scaffold/graft/prosthesis of tracheobronchial [173-183], esophageal [184-188], nerve [189,190], ligament [191-193] and vascular prosthesis [194-199] have been used to numerous patients. However, systematic investigations of colorectal stents made from braided and knitted structures using bio-functional materials are still limited. Until now, design and manufacture of the colorectal stents with good mechanical and biological performance remain a great challenge for material and medical specialists. It requires creativity and inspiration for the design and optimization of stent structures to simulate human colorectal functions. In addition, it is necessary to analyze and design stents by scientific models, including mathematical and animal models.

3.3 Modifications and biotechnology

The surface properties of materials in contact with biological systems play a key role in determining the outcome of biological-material interactions. Surface modification is a unique method to modify and optimize the surface properties of materials and components [200]. There are a multitude of surface modification methods available to engineer custom designed interfaces, including micro and nanostructuring of surfaces via various lithographic techniques, imprinting and laser micromachining; shot peening, laser ablation, plasma spraying, gas plasma treatments, ion bombardment, chemical etching, chemical and physical vapor deposition, as well as coatings of polymers, ceramics, metals, molecular self-assembled coatings, as well as biopolymeric coatings of proteins, sugars, lipids, polyelectrolyte coatings and more several surface modification methods [201,202]. The optimal surface will vary depending on the particular application, such as location of use for medical implants. More significantly, the optimal surface will vary with time, raising an

interest in dynamic surfaces, as well as smart surfaces which react to a changing local biological environment. So, modifications and biotechnology may be facilitates to better biocompatible colorectal stents.

3.4 Biomechanical properties

The mechanical properties play an important role in the colorectal stents practical application, but few studies have been conducted on this aspect about how to optimize its structure and process [203-205]. Dumoulin et al. [206], Francesco et al. [207] used FEM to set up the 3-D models of tracheal stents, and attempted to simulate the stress distribution. The work by Kawaguchia et al. [208] also concentrated on the fabrication of mesh-type knitted structural tracheal Prostheses only, with PP for composite reinforcement.

However, the colorectal stents using the above approaches are scarce. In particular, what is missing is a precise mechanical analysis on the structures and process using mathematics models, offering no basis and effective methods for the application and optimization of colorectal stents.

4. Conclusion

As current operative and non-operative therapies have evolved and improved, the management of CRC is more complex and dependent upon a cooperative, multidisciplinary team approach, particularly in the face of advanced disease. It is the multidisciplinary field involving biology, medicine, material engineering and mathematics that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function.

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Haemostatic Effect of Neutral Wool Hydrolyzed Polypeptides

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Abstract: Neutral Wool hydrolyzed polypeptide (NWHP) is a new biomaterial derived from natural wool. This study was designed to explore the haemostatic effect of NWHP by using different animal bleeding models. The results indicated that wool hydrolyzed polypeptide obviously shortens bleeding time. The NWHP is capable of producing haemostatic effect.

Keywords: neutral wool hydrolyzed polypeptide; haemostatic effect; bleeding model

1. Introduction

Wool is primarily (85-95%) composed of keratin protein which is composed of 19 amino acids constituted by five elements: carbon, hydrogen, oxygen, nitrogen, and sulphur, linked together in a ladder-like polypeptide chains by peptide bonds [1].

Wool keratin and its derivatives are widely used in textile, cosmetic industries. In textiles industries, wool keratin solution is also used as finishing agent for cotton fabric [2]. In cosmetic industries, wool is used because of desirable properties such as strength, insolubility and moisture regain. Wool keratin could improve compatibility, feel, moisturisation and elasticity of skin, wool keratin and its hydrolysates have long been used in skin and hair personal care products [3-4].

Nowadays, wool polypeptide has its new applications in biomedical field. For example, wool material including hydrolysates and powders can provide a rich resource for the production of modified keratin-based biomaterials [5]. Furthermore, Yang et al utilize the extracted water-soluble wool keratin as the building block for the layer by layer (LbL) assembly, which is helpful to prepare a biocompatible surface for tissue engineering [6].

In our previous study [7], the composite amino acid hydrolysate was produced by alkaline hydrolysis of wool. By using ion exchange technique, the alkaline, neutral and acidic amino acids were separated from the composite hydrolysate, and their corresponding dry powders were collected by spray drying.

The aim of this work is to explore the haemostatic effect of Neutral Wool hydrolyzed polypeptide (NWHP) by using different animal bleeding models.

2. Materials and methods

2.1 Materials

NWHP was supplied by Institute of Textile and Clothing, POLYU Hong Kong. It was prepared from natural wool by alkaline hydrolysis. Then by using ion exchange technique, the alkaline, neutral and acidic amino acids were separated from the composite hydrolysate, and their corresponding dry powders were collected by spray drying [7].

2.2 Animals bleeding model

2.2.1 Rabbit ear margin vein bleeding model

New Zealand White rabbits weighing 1.8-2.0 kg are obtained from Experimental Animal Center of Xi'an Jiaotong University in Xi'an, China. All rabbits selected for the study must be in good health; any rabbit exhibiting snuffles, hair loss, loose stools, or apparent weight loss is rejected and replaced. All animals were housed in solid-bottomed polycarbonate cages in SPF animal laboratory with a temperature 21-25°C and a relative humidity of 40-60%. Animals were acclimatized at a 12 h light / 12 h dark cycle and fed a standard diet and tap water ad libitum for a week before the experiments. Experiments were performed in accordance with the Animal Experimentation Committee Regulation.

The animals were randomly divided into 3 groups (NWHP group, Negative control and Positive control) of 5 males and 5 females per group. Physiological saline and Yunnan White Drug-Powder were given as a negative control and a positive control, respectively.

Bleeding model was formed in right marginal ear vein with a razor blade. Bleeding time was measured using a stopwatch from the time of puncture until the time bleeding stopped and was determined by the same person.

2.2.2 Rat tail bleeding model

Healthy Sprague-Dawley rats weighing 220-250g are obtained from Experimental Animal Center of Xi'an Jiaotong University in Xi'an, China. All animals selected for the study must be in good health; any rabbit exhibiting sniffles, hair loss, loose stools, or apparent weight loss is rejected and replaced. All animals were housed in solid-bottomed polycarbonate cages in SPF animal laboratory with a temperature 21-25°C and a relative humidity of 40-60%. Animals were acclimatized at a 12 h light / 12 h dark cycle and fed a standard diet and tap water ad libitum for a week before the experiments. Experiments were performed in accordance with the Animal Experimentation Committee Regulation.

The animals were randomly divided into 3 groups (NWHP group, Negative control and Positive control) of 5 males and 5 females per group. Physiological saline and Yunnan White Drug-Powder were given as a negative control and a positive control, respectively.

Bleeding model was formed in rat tail transected by surgical scissors. Bleeding time was measured using a stopwatch from the time of puncture until the time bleeding stopped and was determined by the same person.

2.3 Statistical analysis

All statistical analyses were carried out using SPSS statistical software version 13.0 (SPSS Inc, Chicago, IL, USA). Data on bleeding time were analyzed using one-way ANOVA. $P < 0.05$ was set as significant.

3. Results

3.1 Rabbit ear margin vein bleeding model

The rabbit ear margin veins were injured in all 30 animals and significant bleeding occurred in each case (Figure 1). The damaged vein in each animal was also similar in depth and site, respectively.

The mean bleeding times in NWHP group were 162 s, ranging from 108 to 205 s (Table 1). Compared with physiological saline group, the bleeding time in NWHP was significantly reduced ($P < 0.05$), but still longer

than that of Yunnan White Drug-Powder group ($P < 0.05$).



Figure 1 Rabbit ear margin vein bleeding model.

Table 1 Effect of NWHP on bleeding time in rabbit ear margin vein bleeding model

Group	Bleeding time	
	Mean \pm SD	Range
Physiological saline	77.40 \pm 22.99	44—119
Yunnan White Drug	214.30 \pm 40.09	149—285
NWHP	162.00 \pm 28.03 ^{a, b}	108—205

^a $P < 0.05$, compared with Physiological saline group

^b $P < 0.05$, compared with Yunnan White drug powder group

3.2 Rat tail bleeding model

The Rat tails were injured in all 30 animals and significant bleeding occurred in each case (Figure 2). The damaged tail in each animal was also similar in depth and site, respectively.



Figure 2 Rat tail bleeding model.

The mean bleeding times in NWHP group were 162s, ranging from 108 to 205 s (Table 1). Compared with

physiological saline group, the bleeding time in NWHP was significantly reduced ($P<0.05$), but still longer than that of Yunnan White Drug-Powder group ($P<0.05$).

Table 2 Effect of NWHP on bleeding time in rat tail bleeding model

Group	Bleeding time	
	Mean \pm SD	Range
Physiological saline	69.70 \pm 17.85	39–96
Yunnan White Drug	199.70 \pm 29.79	152–247
NWHP	134.70 \pm 29.84 ^{a, b}	93–186

^a $P<0.05$, compared with Physiological saline group

^b $P<0.05$, compared with Yunnan White drug powder group

4. Discussion

Neutral Wool hydrolyzed polypeptide (NWHP) is a new biomaterial derived from natural wool.

Our previous study [7] showed that this new material, NWHP have components in crystalline form, which is quite different from the amorphous form of wool before hydrolysis, The Fourier transform infrared spectroscopy (FTIR) measurement also indicates the chemical changes after hydrolysis. The different characteristics of these hydrolyzed polypeptides have various potential applications as functional biomaterials. Therefore, in this study we explore the haemostatic effect of NWHP by using different animal bleeding models.

Rabbit ear is a favorite object for investigations in the field of microcirculation. Marginal vein in rabbit's ear (v. auricularis posterior) is a large thick vessel, situated superficially and very easily accessible for visual research and control [8,9].

Tail bleeding model is another favorite model for pharmacology or toxicology studies, particularly in early discovery work [10,11].

The present study showed that the mean bleeding times in NWHP group were significantly shorter than that in physiological saline control group in two bleeding animal models. From the above results, we could conclude that that NWHP has a haemostatic potential. According to the phenomena that we observed in the process of animal experiment, we presumed that hemostasis is achieved through extremely rapid adsorption of water content in the blood or activates the coagulation factor of body. The detailed mechanisms are worthy of further investigation.

Wound care is a specialized field with a very long history. The speed of stopping bleeding, risk of

infection, speed of healing, and side effects are the four important issues of wound care [12].

Stopping the bleeding is the important step for wound care, it is also an important criteria for judging a wound-care product. So NWHP is a potential material for developing wound care product.

5. Conclusion

This study indicates that NWHP reduced the bleeding time in vivo. NWHP could be a cost-effective haemostatic agent and material for wound dressing.

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Electric Resistances of 24 Jingluo Starting Points in Idiopathic Infertile Men

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Abstract: Infertility affects 10-15% of couples, male factors account for nearly half of all infertility cases. Although modern diagnostic and therapeutic methods are available, unfortunately, approximately 50% of infertility cases are still unexplained. Traditional Chinese Medicine (TCM) has a comprehensive theoretical framework on the essence of body health status among body parts and internal organ systems. This study was designed to explore the relationship between Electric resistances at 24 Jingluo starting points and male infertility. The preliminary results indicate that compared to the normal male population, the infertile men have obvious different electric resistances values in Fei Jing, Gan Jing, Pi Jing, Wei Jing, Dan Jing, Shen Jing, Pangguang Jing ($P < 0.05$) et al, which may lay down the scientific foundation for the next generation of underclothes bioengineering design.

Keywords: male infertility; electric resistance; jingluo

1. Introduction

Infertility affects 10-15% of couples, Male factors account for nearly half of all infertility cases [1]. Although modern diagnostic methods detect more and more organic causes of infertility, unfortunately, approximately 50% of infertility cases are still unexplained or idiopathic for men [2].

Traditional Chinese Medicine (TCM) is one theory system which considers the human body as a whole and determines the treatment based on pathogenesis obtained from differentiation of symptoms and signs. Thus, TCM has a comprehensive advantage in knowing the essence of body health status [3].

Jingluo is a core principle of TCM, the Jingluo plays a central role in the regulation of human health and vitality [4].

In our previous study [5], we found that Jingluo health status determined by a mathematical model of human sickness symptoms are significantly related to the age and the Jingluo balance status measured by the electric resistance at the starting points of each Jingluo. The preliminary results indicate that development of quantitative TCM model to simulate human health is feasible.

Therefore, this study was designed to measure point resistance of 24 starting points of Jingluo in the hands and feet by Ceping apparatus in idiopathic male

infertility population and normal population, and to further investigate the relationship between Jingluo and male infertility.

2. Materials and methods

2.1 Subject selections

This study was conducted between August 2009 and December 2009 in Reproductive center and Reproductive Hospitals in Shaanxi Province. All studies were approved by the Institutional Review Board and the National Medical Ethics Committee, and consents were obtained from all patients.

Study participants seeking treatment for infertility were recruited from the offices of reproductive specialists. All infertility patients met the accepted medical definition of infertility according to WHO criteria. Each man was questioned about his medical and surgical history and underwent a thorough andrological examination. Karyotype, immunological examination of serum, bacteriological examination of the seminal fluid, seminal markers of duct patency (alpha glucosidase, fructose) was measured, and ultrasonography of male reproductive organs was also performed for all patients.

Finally, 199 infertile patients and 36 fertile men (with at least one child) were examined, and then 61

infertile men were selected for the study as idiopathic infertility samples. They did not have child after having sexual life for more than 2 years without contraceptive, varicocele, cryptorchidism, testicular cancer, immune, infection and biochemical abnormality were all excluded. And their female partners were apparently fertile as indicated by various physical and laboratory examination.

2.2 Measuring point resistance of 24 starting points in hands and feet using Ceping apparatus

Environmental temperature is 20-25°C, and relative humidity is 50-60%. After 30 minutes rest, point resistance of 24 starting points in the hands and feet will be measured by Ce ping Apparatus (ZCS-6, DaoPing Ltd., Nanjing, China) .

2.3 Statistical analysis

Descriptive analysis was performed including general baseline information on demographics.

All statistical analysis were carried out using SPSS statistical software version 13.0 (SPSS Inc, Chicago, IL, USA). Comparison between idiopathic infertility and normal population were analyzed using t test. $P < 0.05$ was set as significant.

3. Results

3.1 Demographics

Totally 61 idiopathic infertile and 36 fertile men were finally enrolled in the study, they are all from Han nationality. The mean age in idiopathic infertile men is 29.92 ± 3.56 , that is not statistically different from age (29.14 ± 2.85) of normal fertile men.

3.2 Point resistance of 24 starting points in hands and feet

Compared with normal male fertile population, the idiopathic infertile men have obvious different electric resistances values in Fei Jing, Gan Jing, Pi Jing, Wei Jing, Dan Jing, Shen Jing, Pangguang Jing ($P < 0.05$) (Table 1).

4. Discussion

As a medical issue, impaired fertility affects approximately 80 million people from all parts of the world [1]. Male factors account for nearly half of all

infertility cases. Although the rates of infertility vary throughout the world (ranging from less than 5% to over 30%), unfortunately, infertility affects more people in developing countries [6].

TCM is one of the oldest healing systems. In 2006, TCM provided care for over 200 million outpatients and some 7 million inpatients, accounting for 10-20% of health care in China [7]. Despite decades of research and integration, the fundamentals of TCM remain largely unchanged and its theories inexplicable to science [8].

Table 1 Point electric resistances in 24 jingluo starting points in hands and feet in idiopathic infertile men and normal fertile men

Jingluo	Idiopathic infertile men (n=61)	Fertile men (n=36)	P
Feijing (L)	4.06 ± 0.85	3.33 ± 0.88	0.000*
Feijing (R)	3.85 ± 0.80	3.48 ± 0.96	0.041*
Dachangjing(L)	3.66 ± 0.68	3.48 ± 0.96	0.395
Dachangjing(R)	3.81 ± 0.77	3.52 ± 0.91	0.358
Xinbaojing(L)	3.85 ± 0.95	3.97 ± 1.02	0.544
Xinbaojing(R)	3.79 ± 0.81	3.53 ± 0.81	0.127
Sanjiaojing(L)	4.11 ± 1.05	3.78 ± 0.90	0.112
Sanjiaojing(R)	3.94 ± 0.96	3.79 ± 0.55	0.390
Xinjing(L)	4.13 ± 1.08	3.77 ± 0.59	0.070
Xinjing(R)	4.07 ± 0.97	3.75 ± 0.75	0.090
Xiaochangjing(L)	3.73 ± 0.85	3.56 ± 0.76	0.332
Xiaochangjing(R)	3.77 ± 0.76	3.72 ± 1.04	0.793
Pijing(L)	2.79 ± 0.79	3.59 ± 0.91	0.000*
Pijing(R)	2.61 ± 0.77	3.44 ± 0.83	0.000*
Ganjing(L)	6.59 ± 2.99	3.69 ± 0.97	0.000*
Ganjing(R)	6.29 ± 2.11	4.01 ± 0.98	0.000*
Weijing(L)	3.23 ± 1.08	3.62 ± 0.79	0.065
Weijing(R)	3.14 ± 0.79	3.61 ± 0.66	0.003*
Danjing(L)	3.29 ± 0.84	3.65 ± 0.74	0.037*
Danjing(R)	3.36 ± 0.89	3.69 ± 0.80	0.070
Shenjing(L)	2.07 ± 0.89	3.32 ± 0.63	0.000*
Shenjing(R)	2.00 ± 0.81	3.21 ± 0.81	0.000*
Pangguangjing(L)	1.43 ± 0.87	3.21 ± 1.09	0.000*
Pangguangjing(R)	1.27 ± 0.77	2.77 ± 0.90	0.000*

* $P < 0.05$, compared with fertile group

This study is the first using objective methods to explore the Jingluo changes in idiopathic male patients.

In this study, we found that the idiopathic infertile men have obvious different electric resistances values in Fei Jing, Gan Jing, Pi Jing, Wei Jing, Dan Jing, Shen Jing, Pangguang Jing from that of normal fertile men. These study results are consistent with the old TCM theory and results of other authors [9-11].

Jingluo is the pathway that regulates symmetry and balance to optimize and maintain human health. Our study further confirmed the electrohydrodynamics characteristics of Jingluo, and these study results may lay down the scientific foundation for exploring new methods for diagnosis and male infertility therapy, and also lay down the scientific foundation for the next generation of underclothes bioengineering design.

5. Conclusion

The preliminary results indicate that compared with normal male population, the infertile men have obvious different electric resistances values in Fei Jing, Gan Jing, Pi Jing, Wei Jing, Dan Jing, Shen Jing, Pangguang Jing ($P < 0.05$) et al, which may lay down the scientific foundation for exploring new methods for diagnosis and therapy male infertility, and also lay down the scientific foundation for the next generation of underclothes bioengineering design.

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