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Abstract

Dressing is designed to be in direct contact with the wound. Main objective of wound dressing are to reduce pain, to apply compression for hemorrhage, to immobilize an injured body part, to protect the wound and surrounding tissue, to protect moist wound healing. So far many wound dressing works have been carried out using different polymers for biodegradable and non biodegradable dressing. In this work bacterial activity of wound dressing produced from PVA and acrylic solution were coated by means of different antimicrobial agents and evaluated using Disc diffusion method. PVA is chosen for non removable wound dressing due to its biodegradable nature, flexibility, tensile and adhesive strength whereas acrylic is chosen for removable wound dressing due to its moist environment and wound friendly nature. Chitosan is used as antimicrobial agent to reduce bleeding in wound; honey to release hydrogen peroxide while in contact with wound whereas silver compounds contains more antibacterial activity.

Keywords: Antimicrobial activity, PVA, Acrylic, Chitosan, Honey, Silvernitrate.

1. Introduction

Electrospinning is renowned technique due to its unique properties, high surface area to volume ratio, film thinness, porosity of structure, lighter weight. Nanofibres obtained from electrospinning have diverse applications in nanocomposites such as scaffolding, biomedical and drug delivery system.

Polyvinyl alcohol (PVA) is a water soluble polyhydroxy polymer, which has good mechanical properties, chemical resistance and biological properties. PVA has been electrospun into nanofibers with diameters ranging from 100 nm to 1000 nm. Studies showed that lower concentration of the solution will form fibers with beads and with increment in solution concentration, the form changed from beaded fiber to smooth and uniform fibers [1, 11, 13, and 14].

Acrylic is a long chain synthetic polymer composed of at least 85% by weight of acrylonitrile units [-CH2-CH (CN)-] which resist moths and bacteria.

Chitosan is a polycationic antimicrobial agent. The use of chitosan is limited because of its insolubility in water, high viscosity, and tendency to coagulate with proteins at high pH ^[7, 12, 14].

Honey is a traditional topical treatment for infected wounds. Honey is produced from many different floral sources and its antibacterial activity varies with origin and processing. The antibacterial properties of honey include the release of low levels of hydrogen peroxide ^[2, 6].

Silver has been used as an antimicrobial from 19th century. Silver nitrate is an inorganic compound with chemical formula AgNO₃, a colorless crystalline material that is soluble in water. Silver has antiseptic, antimicrobial, anti-inflammatory properties and is a broad spectrum antibiotic ^[4, 5].

2. MATERIALS AND METHODS:

2.1 Materials:

Polyvinyl alcohol [-CH₂CHOH-]n, 87 – 90 % hydrolyzed, average molecular weight 30000 – 70000 was used for the study. Dimethylformaide solution used as a solvent to dissolve acrylic. Chitosan is insoluble in water but soluble in dilute acids. So 98 % water and 2% acetic acid is used to dissolve chitosan completely. Neem honey was used for this study as antimicrobial agents. Silver nitrate [AgNO₃] with molecular weight 169.87 was used for this study.

2.2 Methods:

2.2.1 Electrospinning:

By using electrospinning concept in this study we produced two different nanofiber webs using PVA for non removable wound dressing and acrylic for removable wound dressing.

2.2.2 Preparation of acrylic nanoweb

Acrylic solution was prepared by mild stirring at 75° C using Dimethylformamide as a solvent to dissolve acrylic. Maximum concentration of solution used to prepare continuous web without beads is 10%. The acrylic solution was fed into syringe fitted with needle tip. The solution was then delivered through a syringe pump at a flow rate of 0.75 mL/h. The distance between the syringe tip and the collector was 10 cm, and a DC voltage of 18 kV was applied between the syringe tip.



2.2.3 Preparation of PVA nanofiber

PVA solution was prepared by mild stirring at 80°C using water as a solvent. PVA solution was fed into syringe fitted with needle tip and delivered at a flow rate of 0.75 mL/h. The distance between the syringe tip and the collector was 10 cm, and a DC voltage of 17 kV was applied between the syringe tip and collector.

2.3 Antimicrobial Agents

In this work three antimicrobial agents are used to analyze the bacterial activity of different biodegradable and non biodegradable polymers.

2.3.1 Preparation of chitosan for coating

Chitosan is available in different forms such as solution, flake, fine powder, bead and fiber. In this study we used high molecular weight chitosan powder with different concentrations such as 0.5, 1, 1.5, 2 %. While increasing the concentration of chitosan the web becomes more brittle so it cannot be used for wound dressing. Chitosan is soluble in dilute acids so 2% acetic acid is used to dissolve chitosan. For PVA, chitosan coating is not used because PVA is hydrophilic in nature.

2.3.2 Preparation of Silver Nitrate coating

Silver nitrate is completely soluble in water partially soluble in acetone. For acrylic nanofibre silver nitrate soluble in water is used whereas for PVA nanofibre, silver nitrate dissolved in acetone and DMF is used due to its water soluble nature.

2.4 Coating Method

There are different methods of coating. In this study we used dip coating. The web is immersed in the bath containing antimicrobial solution for 5 minutes and then

placed in the incubator at 37°C for drying. Drying duration for chitosan and silver nitrate coating is 3 hours and for honey is 48 hours.

2.5 Evaluation of Antimicrobial activity

There are different methods to find the antimicrobial activity. In this study we used Disc Diffusion Method. This method was carried out by three steps. 1) Preparation of nutrient broth 2) Preparation of nutrient agar and 3) Antibacterial test

2.5.1 Preparation of nutrient broath

According to AATCC 147, 2.5 g of nutrient broth was dissolved in 100 ml of distilled water that was taken in a conical flask. The conical flask was closed completely by using cotton plug and then sterilized. In this study we used E. Coli bacteria which is a gram negative. After sterilization, slant bacteria was put inside the conical flask and plugged with cotton. The conical flask was kept inside the incubator for 24 hours at 37 °C.

2.5.2 Preparation of nutrient agar

Another conical flask with 100 ml distilled water was taken with 3.1 g of agar and 2.5 g of broth. This conical flask was closed by means of cotton plug and then sterilized. A negligible quantity of nutrient was poured in petri plate. A swab stick was taken and dipped in the nutrient that contained the bacterial cells. The nanofibrous matrix was placed in prepared Petri plate and antibacterial activity was analyzed from inhibition zone.

2.5.3 Antibacterial test

The microbial suspension was spread evenly over the face of the sterile agar plate. After 24 hours, zone of inhibition appears around the sample. The zone of inhibition was measured from the area on the agar plate that remains free from microbial growth. Twenty eight different samples were produced from biodegradable, non biodegradable polymers were used to determine the antimicrobial activity in Escherichia coli. Table 2 shows that the area of zone formed for different samples. It is found that combination of three antibacterial agents shows higher zone of inhibition value.

3. Results and discussion

In this study we produced twenty eight samples using biodegradable, non biodegradable polymers and different antimicrobial agents. Weights add – on percentage for twenty eight samples are shown in the table 1. When honey is added to silver nitrate, redox reaction takes place which can be easily identified by change in the web colour from brown to dark black due to addition of electrons. This colour change is due to the fact that it absorbs light energy equivalent to the difference in its energy level and appears as black.

			Weight	Weight	
			in g	in g	Add - on
			before	after	Add OII
S.NO	Nanoweb	Coating	coating	coating	percentage
1	IVAIIOVED	0.5 % chitosan in dil. acetic acid	0.026	0.054	107.69
2		1.0 % chitosan in dil. acetic acid	0.035	0.075	114.84
3		1.5 % chitosan in dil acetic acid	0.038	0.09	136.84
4		2.0 % chitosan in dil. acetic acid	0.019	0.084	342.10
5		0.5 % chitosan in dil. acetic acid and dil. honey	0.027	0.136	403.70
6		1.0 % chitosan in dil. acetic acid and dil. honey	0.024	0.127	429.70
7		1.5 % chitosan in dil. acetic acid and dil. honey	0.03	0.157	423.33
8		2.0 % chitosan in dil. acetic acid and dil. honey	0.036	0.22	511.11
9		0.5 % chitosan in dil. acetic acid and conc. honey	0.014	0.066	371.42
10		1.0 % chitosan in dil. acetic acid and conc. honey	0.023	0.116	404.34
11		1.5 % chitosan in dil. acetic acid and conc. honey	0.036	0.192	433.33
12	ACRYLIC	2.0 % chitosan in dil. acetic acid and conc. honey	0.034	0.186	447.05
		0.5 % chitosan in dil. acetic acid , dil. honey and			
13		AgNo3	0.118	0.054	-54.23
		1.0 % chitosan in dil. acetic acid , dil. honey and			
14		AgNo3	0.111	0.049	-55.86
		1.5 % chitosan in dil. acetic acid , dil. honey and			
15		AgNo3	0.126	0.055	-56.34
		2.0 % chitosan in dil. acetic acid , dil honey and			
16		AgNo3	0.152	0.063	-58.55
		0.5 % chitosan in dil acetic acid ,conc honey and			
17		AgNo3	0.041	0.028	-31.71
		1.0 % chitosan in dil. acetic acid , conc honey			
18		and AgNo3	0.096	0.059	-38.54
		1.5 % chitosan in dil acetic acid ,conc honey and			
19		AgNo3	0.153	0.091	-40.52
20		2.0 % chitosan in dil acetic acid ,conc honey and	0.402	0.050	10.16
20		AgNo3	0.102	0.059	-42.16
21		Diluted honey	0.01	0.058	480.00
22		Concentrated honey	0.01	0.07	600.94
23		Silvernitrate	0.019	0.037	94.73
24		Honey	0.032	0.3	837.50

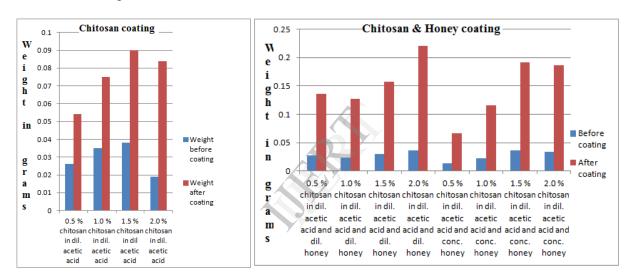
25	POLYVINYL	Silvernitrate (DMF)	0.04	0.104	160.00
26	ALCOHOL	Silvernitrate (acetone)	0.018	0.092	411.11
27		Honey and Silvernitrate (DMF)	0.118	0.103	-12.70
28		Honey and Silvernitrate (acetone)	0.087	0.016	-81.60

The formula used for weight add on percentage

Weight of the material after coating (mg) – Weight of the material before coating (mg)

Weight of the material before coating (mg)

Graph 1, 2, 3 shows change in weight when acrylic coated with different antimicrobial agents.



Graph 4 shows change in weight when PVA coated with different antimicrobial agents.

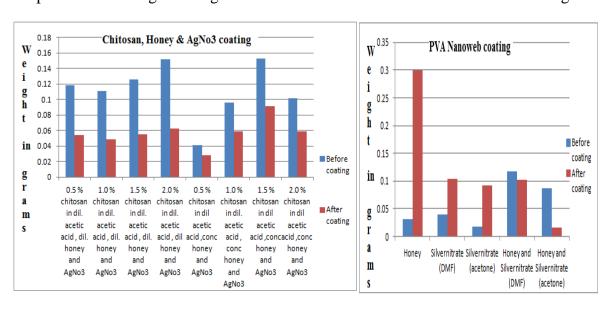
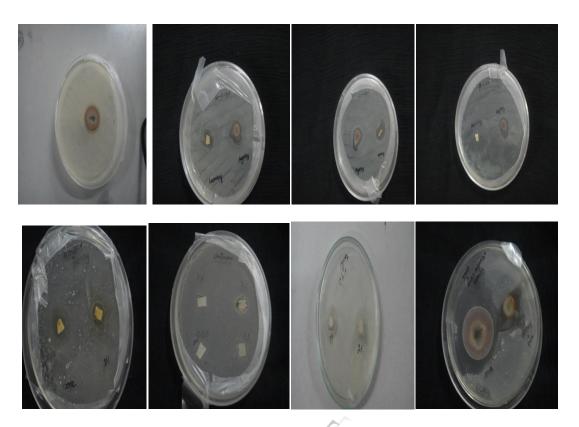


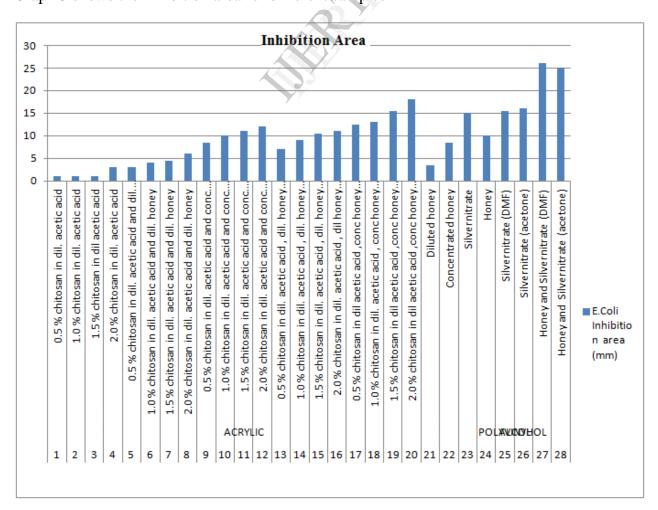
Table 2 shows the antibacterial activity zone for different sample

			E.Coli
			Inhibition
			area
S.NO	Nanoweb	Coating	(mm)
1		0.5 % chitosan in dil. acetic acid	1
2		1.0 % chitosan in dil. acetic acid	1
3		1.5 % chitosan in dil acetic acid	1
4		2.0 % chitosan in dil. acetic acid	3
5		0.5 % chitosan in dil. acetic acid and dil. honey	3
6		1.0 % chitosan in dil. acetic acid and dil. honey	4
7		1.5 % chitosan in dil. acetic acid and dil. honey	4.5
8		2.0 % chitosan in dil. acetic acid and dil. honey	6
9		0.5 % chitosan in dil. acetic acid and conc. honey	8.5
10		1.0 % chitosan in dil. acetic acid and conc. honey	10
11		1.5 % chitosan in dil. acetic acid and conc. honey	11
12	ACRYLIC	2.0 % chitosan in dil. acetic acid and conc. honey	12
13		0.5 % chitosan in dil. acetic acid , dil. honey and AgNo3	7
14		1.0 % chitosan in dil. acetic acid , dil. honey and AgNo3	9
15		1.5 % chitosan in dil. acetic acid , dil. honey and AgNo3	10.5
16		2.0 % chitosan in dil. acetic acid , dil honey and AgNo3	11
17		0.5 % chitosan in dil acetic acid ,conc honey and AgNo3	12.5
18		1.0 % chitosan in dil. acetic acid , conc honey and AgNo3	13
19		1.5 % chitosan in dil acetic acid ,conc honey and AgNo3	15.5
20		2.0 % chitosan in dil acetic acid ,conc honey and AgNo3	18
21		Diluted honey	3.5
22		Concentrated honey	8.5
23		Silvernitrate	15
24		Honey	10
25	POLYVINYL	Silvernitrate (DMF)	15.5
26	ALCOHOL	Silvernitrate (acetone)	16
27		Honey and Silvernitrate (DMF)	26
28		Honey and Silvernitrate (acetone)	25

From this study we analyzed that when three antimicrobial agents are added together the zone is greater but there is a weight loss due to redox reaction between honey and silvernitrate. There are few images which represents the inhibiton area of different samples.



Graph 5 shows the inhibition area for different samples



4. Conclusion

Biodegradable and Non biodegradable nanofibres were produced using electrospinning. Nanofibres were coated by means of different antibacterial agents and analyzing the zone of bacterial activity. From this study we conclude that silver nitrate has high antibacterial activity compared to honey and chitosan but weight loss takes place when honey and silver nitrate combined together due to redox reaction. When three antibacterial agents are used together the bacterial activity is high. When chitosan is coated with nanofibre the result obtained is low due to disturbance of metabolism cells in E.Coli bacteria. For PVA, chitosan cannot be used due to PVA's water soluble nature and acetone and DMF is used to dissolve silvernitrate for coating. PVA has more antimicrobial zone compared to acrylic.

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