Development of Novel Bio functional Polymer-based Natural Biomaterial Films and Surfaces for Biomedical Applications

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A thesis submitted in partial fulfillment of the requirement for the degree of Masters of Science

In
Biomedical Sciences and Engineering

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MASTER THESIS WORK

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DECLARATION

It is hereby declared that this research study has been done for partial fulfillment of requirements for the degree of Master of Sciences in Biomedical Sciences. This work has not been taken from any publication. I hereby also declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification in this university or other institute of learning.

Bakhtawar Ghafoor

DEDICATION

I dedicate my thesis to my parents for their love, encouragement and immense support, to Dr. Abdul Qadeer Khan for making Pakistan to stand with confidence in front of this world by empowering it with nuclear power and to Pakistan Armed Forces for all sacrifices they made for us.

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PUBLICATIONS

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- Fateh Muhammad, Murtaza Najabat Ali, Umar Ansari, Bakhtawar Ghafoor, Misha Mazhar, Salma Mumtaz, Mariam Mir , Automated Suturing Devices in Minimally Invasive Surgeries for Gastrointestinal Tract/Uterine Procedures, Annals of Biomedical Engineering (2015) (Submitted).

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LIST OF ABBREVATIONS

PVA	Poly(vinyl alcohol)
E.coli	*Escherichia coli*
P.aeruginosa	*Pseudomonas aeruginosa*
A.flavus	*Aspergillus flavus*
Spp	Specie
SSIs	Surgical site infections
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared
DDSs	Drug delivery systems
PVAc	Polyvinyl acetate
CipHCL	Ciprofloxacin hydrochloride
PCL	Polycaprolactone
AV	Aloe vera
PVP-I	Poly-vinyl-pyrrolidone Iodine
PEG	Polyethylene Glycol
HPMC	Hydroxy-propyl-methyl-cellulose
PAA	Poly-acrylic acid
DMF	Di-methyl-formamide
PBS	Phosphate buffer solution
NIH	National Institutes of Health
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide

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ABSTRACT

ABSTRACT

The study focuses on the development of novel Aloe vera based polymeric composite films and antimicrobial suture coatings. Poly-vinyl alcohol (PVA), a synthetic biocompatible and biodegradable polymer was combined with Aloe vera, a natural herb used for soothing burning effects and cosmetic purposes. The properties of these two materials were combined together to get additional benefits such as wound healing and prevention from surgical site infections. PVA and Aloe vera were mixed in a fixed quantity to produce polymer based films. The films were screened for anti-bacterial and anti-fungal activity against bacterial (*E.coli, P.aeruginosa)* and fungal strains *(Aspergillus flavus* and *Aspergillus tubingensis)* were screened. Aloe vera based PVA films showed antimicrobial activity against all the strains; the lowest Aloe vera concentration (5%) showed the highest activity against all the strains. In-vitro degradation and release profile of these films was also evaluated. The coating for sutures was prepared, *in-vitro* antibacterial tests of these coated sutures were carried out and later on *in vivo* studies of these coated sutures were also performed. The results showed that sutures coated with Aloe vera/PVA coating solution have antibacterial effects thus have the potential to be used in the prevention of surgical site infections and Aloe vera/PVA based films have the potential to be used for wound healing purposes.

Key words: Aloe vera, PVA, drug release profile, *in vivo* studies and coated sutures.

1. INTRODUCTION

Nosocomial Infections are **hospital-acquired infections** (**HAI**) that usually develop in patients during their hospital stay, affecting the health expenditure of the patient (Nautiyal et al., 2015). The main factors that make patients prone to nosocomial infection include concurrent infections, medical devices, surgery, immunosuppressive agents and emergence of multidrug resistant pathogens (Lahsaeizadeh, Jafari, & Askarian, 2008). Pathogens are responsible for such infections are known as Nosocomial Pathogens. Among them 90% bacterial pathogens are involved, however mycobacterial, viral, fungal or protozoal agents are less commonly involved (Taylor, Buchanan-Chell, Kirkland, McKenzie, & Wiens, 1997). According to the data, *Escherichia coli*, *Staphylococcus aureus*, *enterococci* and *Pseudomonas aeruginosa* are the most common nosocomial pathogens (Horan, Andrus, & Dudeck, 2008). Among the fungal pathogens, *Candida albicans* (Banerjii, 1991), Aspergillus spp., and especially *Aspergillus fumigatus, A. flavus, and A. terreus* have also been reported as the common cause of nosocomial infection in highly immunocompromised patients. These pathogens can be transmitted either through inhalation or direct contact with occlusive materials (Bodey, 1988; Fridkin & Jarvis, 1996) .

One of the most common nosocomial infections are surgical site infections (SSIs) mainly caused due to infected suture materials used in surgery and medical implants (C. D. Owens & K. Stoessel, 2008). These infections are usually difficult to resolve and may cause complications in extreme cases. In order to prevent surgical site infections, scientists have been using several natural and synthetic materials like plant extracts and polymers which may be used as coating materials on surface of medical devices such as surgical implants or sutures (Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011). The addition of antibiotics to these coating biomaterials can provide the local delivery of antibiotic directly at implantation or suture site, thereby

decreasing the onset of infection (Goldstein, Levy, Labhasetwar, & Bonadio, 2005). Synthetic and natural biomaterials have also been used in other biomedical applications such as drug delivery systems, wound infections, antitumor and anti-inflammatory agents (Cascone, Sim, & Sandra, 1995).

Among synthetic biomaterials, one of the extensively used polymers is poly (vinyl alcohol) (PVA). Due to its suitable chemical and physical properties, biocompatibility, biodegradability, easy preparation and nontoxic nature, PVA has been studied intensively in different biomedical applications including wound dressings, contact lenses, coatings for sutures and catheters (Walker, Young, Hunt, & Henderson, 2007; Yang, Lee, Lin, Yang, & Chen, 2007).

Aloe vera, as a natural source of bioactive compounds, is widely studied for biomedical applications. Aloe vera belongs to the *liliaceae* family and is known as the oldest therapeutic herb. It has the ability to promote wound healing as well as to treat burn areas on the skin (SCHMIDT & GREENSPOON, 1991; Wani, Hasan, & Malik, 2010). Due to its properties, many researchers have shown the antibacterial, antiviral, antitumor and anti-inflammatory activity of different parts of Aloe vera such as its stem, root and leaf extracts (Hamman, 2008; Pandey & Mishra, 2010; Reynolds & Dweck, 1999). The chemical composition of Aloe vera has also proved its potential use in cosmetic formulations, food supplements and medical devices (Hamman, 2008; M. H. Radha & N. P. Laxmipriya, 2015; Silva, Caridade, Mano, & Reis, 2013).

The present work focuses on the antibacterial and antifungal activity of Aloe vera/PVA composite membranes and the application of these blends in the prevention of nosocomial infections; for the specific purpose of investing this, sutures coated with the PVA/Aloe gel blend have been used for both *in-vitro* and *in-vivo* analysis. Aloe vera/PVA films have been characterized through SEM and FTIR analysis. The *in vitro* degradation and drug release profile test of the blend films is also evaluated.

2. LITERATURE REVIEW

One of the most common types of nosocomial infections is surgical site infections as they occur at the surgical site. This occurrence of SSIs contributes to the high cost of medical facilities and also results in high mortality in patients (Broex, Van Asselt, Bruggeman, & Van Tiel, 2009; C. Owens & K. Stoessel, 2008). Surgical site infections (SSIs) are still a very problematic area in the field of medicine and surgery. Because of the fact that technology has advance to very high level and there are hundreds of ways to prevent infections but still the rate of SSIs is still very high (M. L. Storch, Rothenburger, & Jacinto, 2004). The SSIs result in additional stay of patient in hospital with additional financial load and pain that patient suffers from because of SSIs (Mingmalairak, Ungbhakorn, & Paocharoen, 2009; Rucinski, Fabian, Panagopoulos, Schein, & Wise, 2000).

The reason of occurrence of infection during surgery is because few numbers of bacteria attached to medical devices used during surgical processes remained viable despite of the action of antimicrobial agent (Chuard et al., 1991; Kaiser, Kernodle, & Parker, 1992). Studies have been carried out to validate the claim that bacteria attached to medical devices remain viable and escape host defense system also from the antimicrobial agent used to sterilize the equipment. Results proved that even the strains which are not much efficient in producing the infection also remain active and cause the infections. And the sources of introducing these bacterial strains into the body or surgical area are medical devices (Vaudaux et al., 1992; Zimmerli, Waldvogel, Vaudaux, & Nydegger, 1982).

Furthermore, the source of introducing the sufficient number of bacteria into the site to cause SSI is suture too and suture material through its capillary action during suturing process also aids in transporting strains to the target sites (C.-C. Chu & Williams, 1984; M. L. Storch et al., 2004). Sutures are thread like structure used to seal blood vessels and tissues. The use of different sutures depend upon the type of

tissue, nature of wound, load to be tolerated by tissue and location where suturing is required (Burg & SHALABY, 1998). They are the most common and widely used material to be used as medical implants. The approximation of the use of suture in USA alone is upto 250 million sutures per year used in different sites. Thus there is need of development of a suture material with antimicrobial property so as to reduce the level of SSIs in patients (Pratten, Nazhat, Blaker, & Boccaccini, 2004).

If the medium of transportation of the bacterial strains is eliminated then risk of infection can be reduced. Because when the medical device is colonized b bacterial colonies than the methods to disinfect the devices become fail as they are unable to decontaminate the devices (Shunmugaperumal, 2010).

There are many approaches to reduce the SSIs. One of them is to coat the medical devices with material that is antimicrobial in nature, which can prevent the bacteria from colonizing the medical devices and results in decrease in bacterial growth at surgical site (Li et al., 2012). The reason of moving towards the coating of the medical devices in order to reduce the risk of introduction of bacterial cells into the surgical site is that, once the bacteria are able to colonize the medical device they form films. Thus preventing antibiotics to penetrate into the infected sites thus leads to an increase in infection (An, Friedman, Draughn, Smith, & John, 1996).

The medical devices coated with antimicrobial agent are helpful in the reduction of the bacterial related infections. The studied were carried out to check the level of infection after implanting triclosan coated graft material in the femoral arteries of animals followed by introduction of bacterial inoculum into the graft area. The results showed positive reduction in bacterial colonizes at graft site in animal model (M. Storch, Scalzo, Van Lue, & Jacinto, 2002). There are many suture coating materials used in present era. For example, Teflon coated polyamide fiber, silicone coated surgical silk suture and poly (vinyl alcohol) coated with different polymer chains having antimicrobial properties. Dines et al., coated the suture with growth factors in order to accelerate the healing of the teared tendon through coated growth factors (Tollar, Štol, & Kliment, 1969).

CHAPTER 2 LITERATURE REVIEW

The small neutral molecule, triclosan is in use now a day as antimicrobial agent. The triclosan can inhibit wide range of bacterial strains. But triclosan has been used in many products such as toothpastes, shower gels, paints, etc. which causes the development of resistance in bacterial strains against triclosan (Schweizer, 2001).

The polyglactin 910 suture is coated with polyglactin 370 coating material, which is absorbable water insoluble coating (C. Chu, 1997). Later on polyglactin 910 was also coated with triclosan (vicryl plus) used to close fascia tissue. The results were in favor of suture coated with triclosan obtained from clinical trials (Mingmalairak et al., 2009).

Aloe barbadensis miller also commonly known as Aloe vera belongs to Liliaceae family and is perennial succulent with hundreds of species (Morton, 1961). It is mostly grown in harsh, hot and dry environment of tropical and subtropical climate like that of USA and South Africa (Eshun & He, 2004). It has pointed long leaves consisting of two parts, a green rind covering the inner clear gel pulp. The gel comprises of major part and volume of the leave (Ni, Turner, Yates, & Tizard, 2004).

The leaves of the Aloe vera contain mucilaginous gel under its parenchymatous tissue. The Aloe gel has been used to treat topical skin wounds and burns since 1959 (Vazquez, Avila, Segura, & Escalante, 1996) (Vazquez et al., 1996). Literature tells us that Aloe vera has anti-inflammatory, UV protective, antioxidant, antimicrobial, anti-immunomodulatory and wound and burn healing properties. Out of these properties of Aloe vera, Aloe vera's wound healing and antimicrobial properties are extensively studied (Vazquez et al., 1996).

For hundreds of years, Aloe vera has been in use as folk medicine and is very popular among India, China, Japan and West Indies cultures. The reason of including Aloe vera as an important component of traditional medicine is because of its high water content comprising of about 99-99.5% of Aloe contents along with remaining components that includes phenolic compounds, organic acids, minerals, vitamins and

enzymes and some secondary metabolites such as anthraquinones (Aloe emodin) and tricyclic aromatic quinines (Maharjan H Radha & Nampoothiri P Laxmipriya, 2015).

During wound healing process, growth of fibroblast cells is accelerated and stimulated by Aloe gel results in increase strength of wound and collagen proliferation in tissue to be healed (Davis, DiDonato, Johnson, & Stewart, 1994; Davis, Stewart, & Bregman, 1992). Furthermore, the increase content of aldehyde present in Aloe vera results in increase crosslinking of tissues and decrease in acid content at wound site which further increase the accumulation and formation of tissue granules accelerating the healing process (Chithra, Sajithlal, & Chandrakasan, 1998; Thompson, 1991). The application of Aloe vera extracted gel over the wound area also increases the angiogenesis resulting in increased supply of blood and nutrients to the wound area (Choi & Chung, 2003).

PVA is a synthetic polymer which is biocompatible and biodegradable, thus it is used in many biomedical applications (Baker, Walsh, Schwartz, & Boyan, 2012). PVA is been used to form different types of blends because of its film forming property and stability. Like polyethylene, PVA has zigzag planar structure. PVA is a hydrophilic synthetic polymer and is soluble in water (Pal, Banthia, & Majumdar, 2007).

PVA has hydroxyl group in its backbone imparting good mechanical and tensile strength to PVA films. PVA is biocompatible and biodegradable because of its hydrophilic nature which allows living cells to interact with PVA. PVA due to its stability, chemical resistance, and nontoxicity, biocompatible and degradable properties is used in many pharmaceutical and biomedical applications (Abdullah, Sekak, Ahmad, & Effendi, 2014; Kim, MICHLER, & PÖTSCHKE, 2010).

PVA is also being used as material for control release of drug. PVA is being loaded with drug and is used in drug delivery systems (DDSs). But because of its solubility in water, it get dissolved in water very easily thus limited its use in DDSs. To overcome this issue, PVA is crosslinked with other polymers which are comparatively stable in water. PVA was co-polymerized with PVAc which is also a

biodegradable and biocompatible polymer and electrospun to form nanofibrous (Jannesari, Varshosaz, Morshed, & Zamani, 2011).

For the accelerating proliferation of epidermal and dermal cells during the process of healing of wound, wound dressing materials are used. The wound dressing material act as barrier against microbial attack, and is permeable to moisture and oxygen helping in the wound healing. The advantages of providing environment required for wound healing like, required moisture and oxygen level, prevention against microbes, providing aseptic conditions at wounded area and removal of all worn out cells from the site of injury, are all provided by the use of wound dressing material such as, collagen, alginate, hydrogels, transparent this films and many more like these (Mogoşanu & Grumezescu, 2014).

The electrospun nanofiber mats of blend of PVA and PVAc loaded with CipHCl were successfully fabricated. The addition of drug decreases the drug release kinetics of the nanofibers and release occurred of longer period of time. While the addition of PVAc results in comfortable and flexible electrospun mats making them easier to be used as wound dressing material which can be placed anywhere (Jannesari et al., 2011).

Pereira et al., using solvent casting method formulated the Aloe vera based alginate films to be used in many fields such as, biomedical and pharmaceutical companies. The addition of Aloe vera results in improved water absorption property of films with the outcome of decrease in film weight loss. The decrease in weight of the film gives the benefit of longer sustain period with slow drug release if drug is incorporated in the films (Pereira et al., 2011).

Kakroodi et al., used natural polymer, cellulose, as a cross linker, in order to investigate the effect of cellulose extracted from Aloe vera rind on the film properties of PVA. The mechanical testing of casted films with PVA and cellulose showed improved tensile strength, greater young modulus with good thermal stability. These properties make cellulose based PVA films a better candidate to be used in biomedical field (Kakroodi, Cheng, Sain, & Asiri, 2014).

The electrospun nanofibrous scaffolds of PCL-AV were manufactured. These scaffolds have the advantage of supporting and proliferation of shin cells. The scaffolds provide a guided growth path to proliferating and growing cells and help them to maintain their phenotypic morphology. The addition of Aloe vera helps in accelerating the regeneration of the skin cells at the site of injury (Suganya et al., 2014).

In the field of tissue engineering, scaffolds are extensively studied and used. A 3D scaffold containing collagen, for providing structural support, chitosan, for providing extranutritional support and Aloe vera for accelerating the renewal and proliferation of cells into the scaffold, was fabricated through electrospinning. The resulting scaffold has all the physiochemical and biological properties which are needed for the tissue engineering purposes (Jithendra, Rajam, Kalaivani, Mandal, & Rose, 2013)

Nanofibers are widely investigated to be used in wound healing properties. PVP/PVA along with chitosan and iodine were electrospun to obtain nanofibers with nano pores. The addition of chitosan in the polymeric blends resulted in increased viscosity and electrical conductivity which contributed to the decreased pore size of the fibers. With the addition of different drugs which can contribute towards the wound healing process will make such nanofibers likely used in wound dressing materials (Gökmeşe, Uslu, & Aytimur, 2013).

The use of hydrogel for wound healing purposes is a very common method. Hydrogels have the property of replacing damaged tissues from the body. Kyong and his colleagues synthesized the hydrogel using PVA/PVP and Aloe vera using freeze thaw and gamma-ray irradiations procedure. The content of Aloe vera affected the physical properties of gel. The decrease level of Aloe vera contributed towards greater gel strength of the hydrogel but with decreased swelling behavior (K. R. Park & Nho, 2004).

In an attempt of fabricating an antimicrobial film with nano pores to allow oxygen and moisture for wound to heal properly and quickly, Ibrahim et al., fabricated

PVA/PVP-I/PEG loaded with Aloe vera and HPMC as drug. The addition of Aloe vera shifted crystal structure to amorphous of fabricated polymer blend films resulting in improved thermal and decomposition properties of blend nano-mats. They used electrospinning to fabricated these films and through electrospinning nano size pores were generated having the capability of prevent entry of microbes to wounded area (Uslu & Aytimur, 2012).

The nanofibers obtained through electrospinning are well been used. PVA/PAA was electrospun followed by the loading of CipHCl and Aloe vera. The porosity obtained after electrospinning provides the advantage of being permeable to oxygen and moisture and non-permeable to bacteria. The addition of Aloe vera along with the drug CipHCl adds the benefit of being a antimicrobial agent with controlled drug release. Thus such films have great scope in wound and burn healing processes (Serinçay et al., 2013).

3. MATERIALS AND METHODS

3.1 Collection of plant material

Fresh Aloe vera plants were collected from local nurseries and the leaves washed well with distilled water to remove all contaminants present at the surface. The gel was harvested from the leaves in an autoclaved container and kept at room temperature for further use.

3.2 Test organisms for *in vitro* and *in vivo* studies

In order to investigate antimicrobial and antifungal activity (*in-vitro* studies), pure cultures of bacterial and fungal strains including *Pseudomonas aeruginosa (P.aeruginosa), Escherichia coli (E.coli),Aspergillus tubingensis* and *Aspergillus flavus* were obtained from Mycovirus Research Lab, National University of Sciences and Technology (NUST) H-12 Islamabad. The pure bacterial and fungal cultures were stored in agar at 4°C.

3.3 Suture Materials

Commercially available silk braided black surgical sutures (1.5 metric, size 4-0) were used to carry out *in vitro* and *in vivo* studies. The suture material was delivered in sterile single peelable foil packages and stored at room temperature. For investigation, the sutures were cut into defined lengths (1 cm) under aseptic conditions.

3.4 Preparation of Aloe vera based PVA films

Polyvinyl alcohol (PVA), a biocompatible polymer, was used for the formation of Polymer/Aloe vera films (Aytimur, Koçyiğit, & Uslu, 2013). Dimethyl Formamide (DMF) was selected as solvent for the formation of PVA-Aloe vera films, due to its high volatility.

Solvent-casting method was used for the fabrication of Aloe vera gel /PVA films. 1 g of PVA was dissolved in 40 ml of DMF. The solution was stirred with a constant RPM at 60°C until PVA was completely dissolved, and a clear solution was obtained. This was followed by the addition of different amounts of Aloe gel. Aloe gel was added in the amounts of 5%, 10%, 15% and 20% respectively, for the fabrication of Aloe/PVA films with varying Aloe gel compositions. The heating was turned off while constant magnetic stirring was continued to obtain a homogenized mixture of Aloe vera gel and PVA in DMF. The mixture was poured into Petri dishes and placed in oven at 37°C for 20 h to evaporate the solvent completely and films were harvested for further testing.

3.5 Antimicrobial testing of Aloe vera based PVA films

The antifungal and antibacterial activities of films were evaluated using disc diffusion method. For antibacterial activity sterile nutrient agar (pH: 7.4) was prepared and poured in petri dishes which were inoculated with the 0.1 ml of bacterial inoculum from pre-culture of test bacterial strains.

For antifungal investigation, sterile potato dextrose agar was prepared and poured onto the petri plates and pure fungal cultures were obtained from test fungal strains.

For disc diffusion test, films were cut into discs of about 7 mm in diameter and placed on the bacterial and fungal inoculated plates with certain distances. Each petri plate contained five discs one of which included the control sterile Whatman filter paper

no.1 and other four Aloe vera/polymer based films with varying concentrations of Aloe vera (5%, 10%, 15% and 20%).

For antibacterial testing a positive control (Tetracycline disc) was used. All plates were incubated at 37°C for 24 h. The zone of inhibition diameter in millimeter (mm) was measured. The study was performed in triplicates and mean was calculated.

3.6 Characterization of Aloe vera based PVA films

3.6.1 Fourier Transform Infrared (FTIR) Analysis

Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, spectrum 100 FTIR spectrophotometer) of Aloe vera/PVA films was carried out (at 256 scans, 8 cm^{-1} resolution) to investigate the presences of functional groups and types of interaction between the Aloe vera and PVA components.

3.6.2 Morphological analysis: SEM

Scanning Electron Microscopy (SEM) was performed to find out the surface morphology of the casted films. The assessment of the surface morphology of the Aloe vera/PVA based films was done using JSM-6490A Analytical scanning electron microscope (JEOL, Tokyo, Japan). SEM images were collected at an activation voltage of 20 KV.

3.7 *In vitro* degradation and drug release profile testing of Aloe vera based PVA film

The degradation profile was assessed by recording weight differences after regular time intervals while drug release profile of Aloe vera/PVA films was assessed

through UV-Vis spectrophotometry. A portion of Aloe vera/PVA films with measurable size (1" by 1") were cut and placed in 3 ml of PBS (pH 7.4) at 37°C. PBS was removed after every 10 minute interval and replaced with fresh 3 ml of PBS. The films were weighed before addition of PBS and after they were taken out of the PBS solution, the weights were subtracted and recorded. Moreover the drained PBS solutions were evaluated for drug release profile by UV-VIS spectrophotometer (Systronics 2202) absorbance at λ max= 301 nm. The degradation and release tests were carried out in triplicates and an average value was calculated.

3.8 Coating of sutures

Dip coating method was used to coat the sutures. For dip coating, the solution was prepared by mixing 2 g of Aloe vera and 1 g of PVA in 40 ml of DMF. The sutures (30 cm length) was first sterilized and then dipped in the dip coating solution (for 60 minutes) followed by removal and air drying of suture for 24 h. The confirmation of coating of the suture was done by measuring the weight before coating and after coating.

3.9 *In vitro* evaluation of coated and uncoated sutures

The silk sutures (with and without Aloe vera/PVA coating) were evaluated *in vitro* antibacterial activity against two bacterial strains that is, *E.coli* and *P.aeruginosa*. Nutrient agar media (pH: 7.4) plates were prepared and the coated suture of the size 4 cm was placed over agar. The plates were than inoculated with bacterial strains (*E.coli and P.aeruginosa*) and anti-bacterial activity was recorded.

3.10 *In vivo* evaluation of coated sutures

BALB/c mice were purchased from National Institute of Health (NIH) for the *in vivo* analysis of coated sutures. To check the antimicrobial activity of the coated sutures *in vivo*, mice were given an incision of about 2 cm on both sides of the spine. The incision was inoculated with *E.coli* (30×10^6 colony forming unit (CFU)) of 100 µl with the help of a syringe. Afterwards, in one incision suture with the coated material was placed and in the other suture without coating material was placed. A discontinuous suturing was done to close the incision site. The same procedure was carried with the mice using *P.aeruginosa* (50×10^6 CFU) of 100 µl for inoculation. The entire experiment was performed in triplicates using sterilized instruments. The sutured incision sites were covered with surgical tape for two days. After two days, sutures from both sides of mice was taken out and placed in separate 1.5 ml centrifuge tubes containing 100 µl PBS solution, the sutures were placed on the petri dishes containing nutrient agar and placed in an incubator at 37°C overnight.

3.11 Statistical analysis

All the quantitative data were expressed as mean value with standard deviation. The Statistical analyses of the results were done by using T-test in Graph Pad Prism 6.0 software. The values that were $P < 0.05$ were considered statistically significant value.

4. RESULTS AND DISCUSSION

4.1 Scanning Electron Microscopy (SEM)

The surface morphology of different films was assessed by SEM which has been demonstrated in Figure 1.The SEM images showed the aggregates of Aloe vera dispersed on the surface of films which contributed to the film surface roughness. Similar results have been reported by Pereira et al., while studying the properties of alginate based Aloe vera films (Pereira et al., 2011).

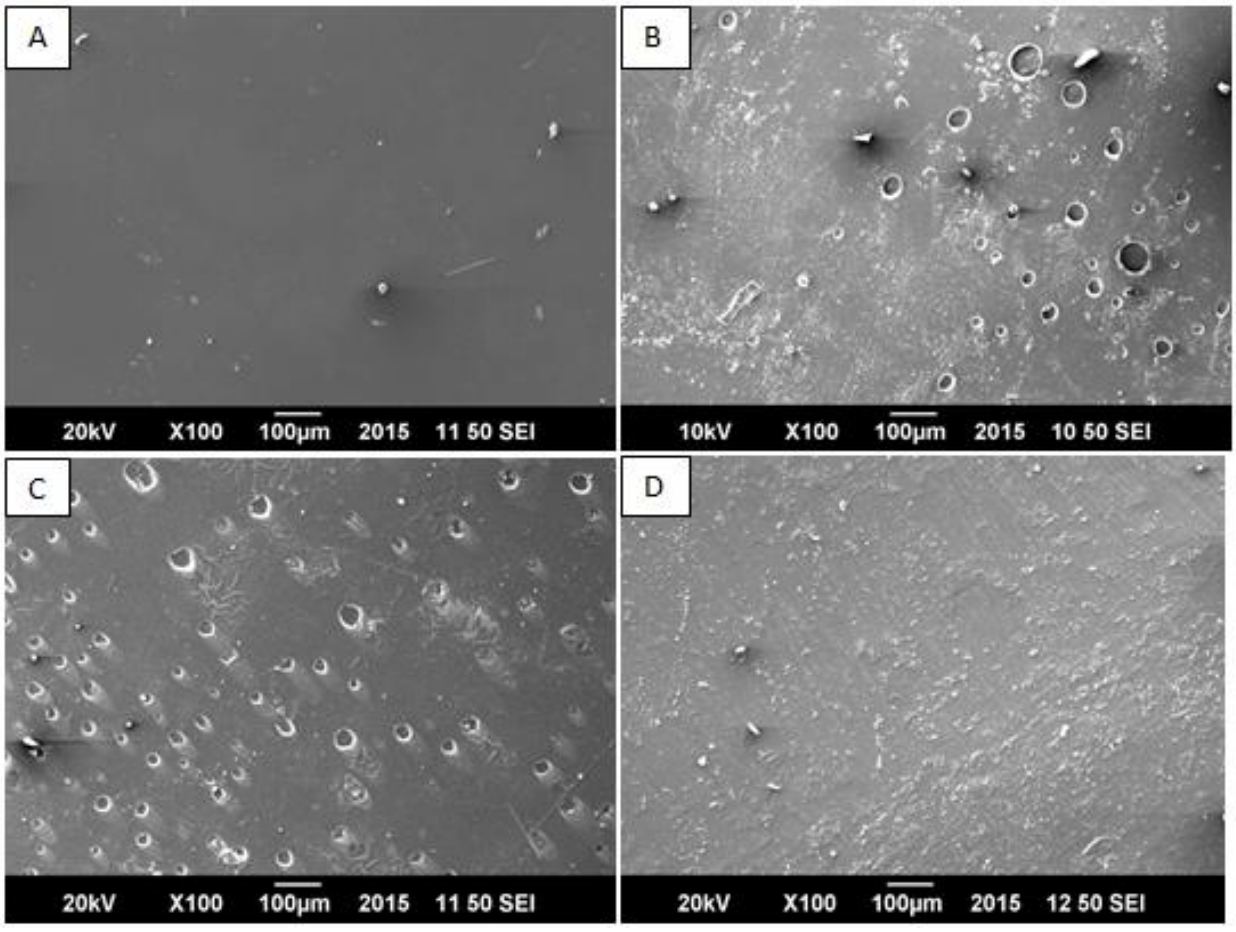


Figure 1: SEM images of Aloe vera/polymer films. (A) Film with 5 % Aloe vera concentration. (B) Film with 10 % concentration. (C) With 15 % and (D) with 20% concentration at 20kV and X100 magnification

4.2 Fourier Transform Infrared (FTIR)

FTIR analysis was performed to identify the nature of linkages between PVA and Aloe vera. The FTIR spectra of Aloe vera/PVA films with varying concentrations have been shown in Figure 2. The peak appeared between 3500 cm^{-1} to 3200 cm^{-1} in all films indicates the presence of hydroxyl group (OH) (Kumar, Gayathiri, Ravi, Kabilar, & Velmurugan, 2011). The absorption band between 3000 cm^{-1} to 2800 cm^{-1} centered at 2932.68 cm^{-1} in 5% Aloe vera/PVA and 2926 cm^{-1} in 20% Aloe vera/PVA. Both peaks had shifted from 2922 cm^{-1}, this was a characteristic of asymmetric stretching of CH_2 groups (Lim & Cheong, 2015). The shift indicated the intermolecular interactions at these functional groups in Aloe vera and PVA. The peaks obtained at the range of 1720 cm^{-1} to 1710 cm^{-1} corresponds to the stretching of C=O group which indicated the presence of carbonyl compounds in Aloe vera. The presence of C-O-C (phenol ether) group was indicated by the bands located at 1036 cm^{-1} in films having 20% Aloe vera/PVA concentration. The peak in pure Aloe vera at 1075 cm^{-1} (Lim & Cheong, 2015) was shifted to 1036 cm^{-1} indicating the presence of C-N functional groups in the films; the shift observed in the peak can be attributed to interactions between amine groups and hydroxyl groups of Aloe vera and PVA respectively (Venkatesh et al., 2015). The absorption band 1460 $cm^{-1}$to 1410 cm^{-1} appeared in all concentrations of Aloe vera/PVA films, hence representing symmetric stretching vibrations of COOH groups in films (Venkatesh et al., 2015). The broad peak at 1150 cm^{-1} to 1130 cm^{-1} could indicate either (C-O) stretching vibrations in films with concentrations of 5% and 15%. The absorption peaks obtained at 860 cm^{-1} to 840 cm^{-1} correspond to rocking vibrations of CH_2 bonds in PVA (Kim et al., 2010). The bending of C-H alkyl groups present in Aloe vera and PVA at a peak range of 950 cm^{-1} to 940 cm^{-1} can be easily be seen in FTIR results. A new peak at 2171.18 cm^{-1} in 5%, 2167.69 cm^{-1} in 15% and 2168 cm^{-1} in 20% Aloe vera/PVA film indicates the occurrence of interactions between CH group of PVA with CH group of Aloe vera. The band at 1660 cm-1 and 1264 cm-1 in 20% Aloe vera/PVA film demonstrated the interaction between hydrogen groups and C-O-C of PVA and C=O

and C-O-C groups of Aloe vera (Abdullah et al., 2014; Kim et al., 2010; Lim & Cheong, 2015).

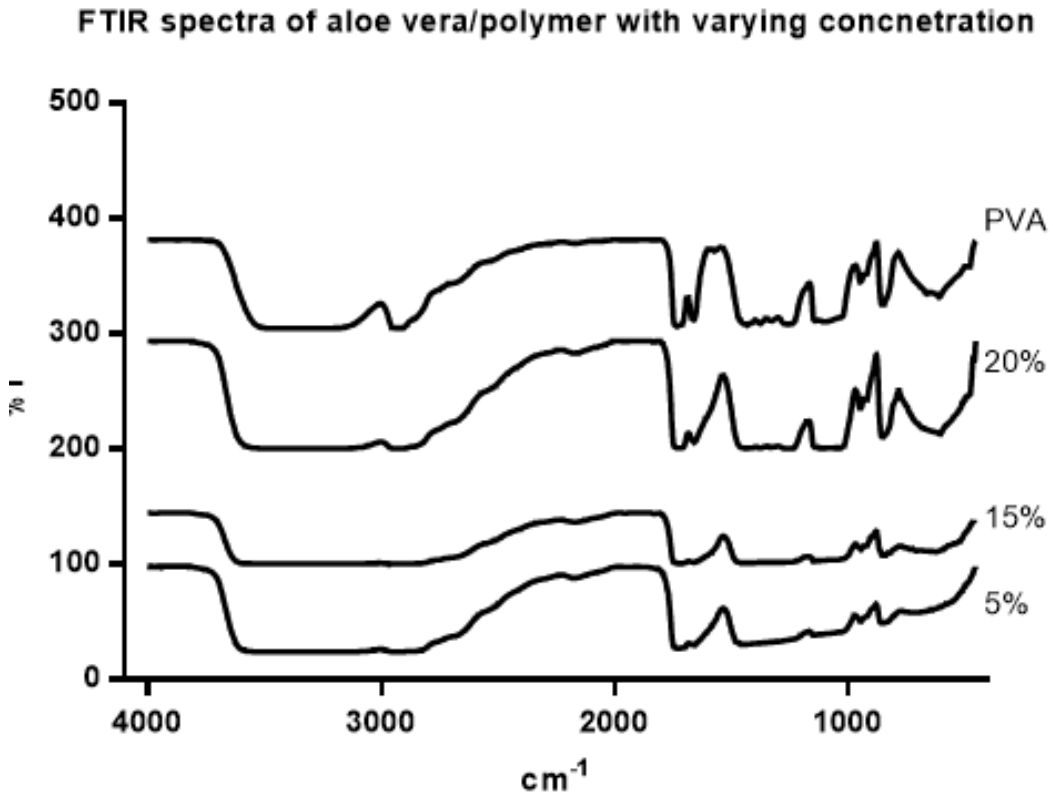


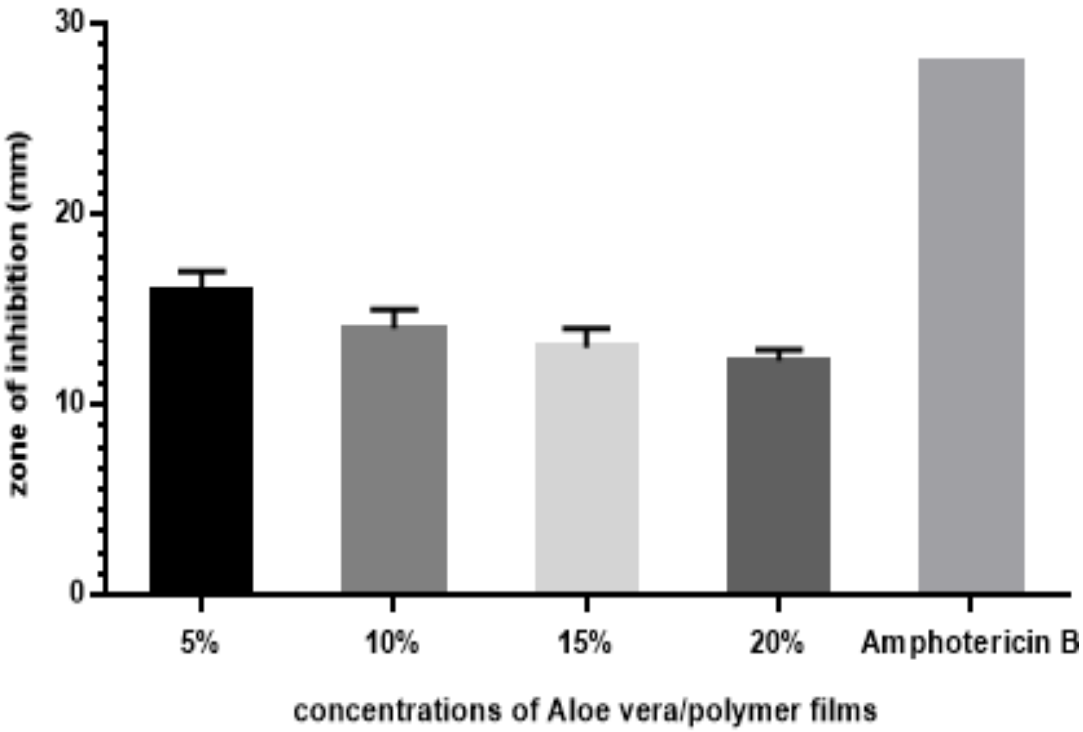
Figure 2: FTIR results of PVA film and Aloe vera/polymer films with 5%, 15% and 20%.

4.3 Antimicrobial testing results of films

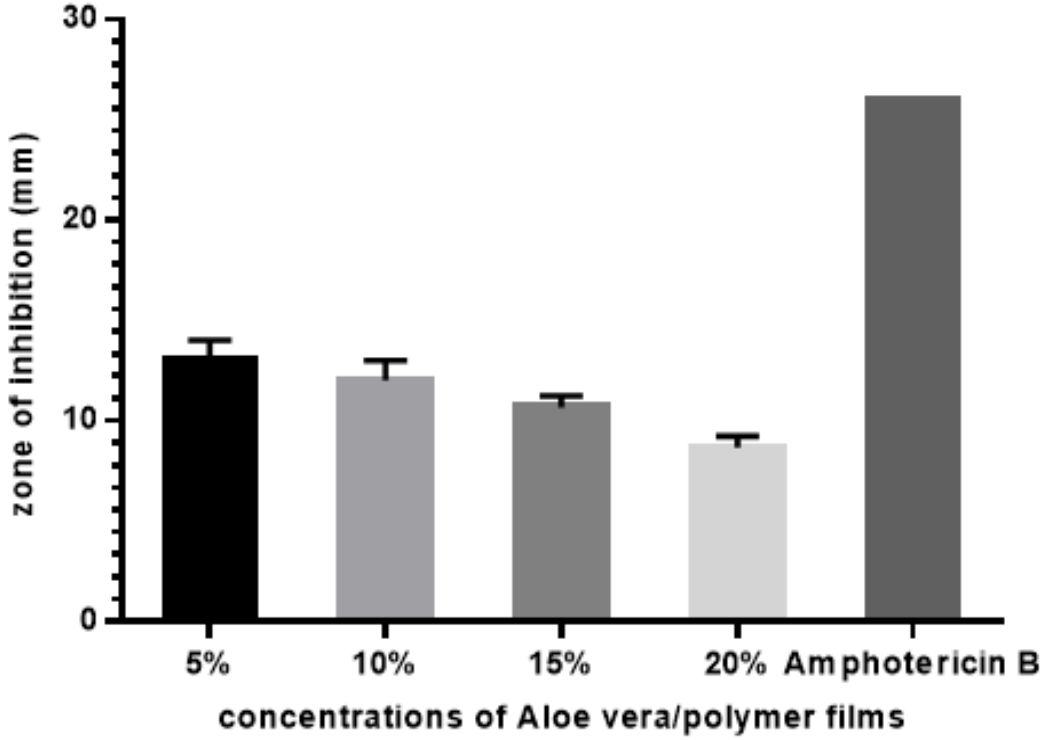
The Aloe vera/PVA films when positioned on bacterial and fungal inoculated plates gave zones of inhibition which were recorded after 24 h of positioning the films (Figure 3). All films demonstrated the antimicrobial activity due to the release of Aloe vera from the surface of the films. The maximum activity was indicated by 5% Aloe vera/PVA combination. The potential reason could be the presence of a lower number of interactions between Aloe vera and PVA; because of lower concentrations

of Aloe gel, they were not chemically bound to each other thus keeping the components and their respective functional groups of Aloe vera chemically active against microbial activity. Increased levels (10%, 15% and 20%) of Aloe vera in the PVA blend lead to the increased interactions between both components which causes the shift in FTIR peak (Figure 2) thus such interactions of Aloe vera/PVA may influence antimicrobial activity. Antonisamy et al., demonstrated the antimicrobial activity of DMSO extracts of Aloe vera gel against human pathogens and highest zone of inhibition (13 mm) against *E.coli* was recorded (Antonisamy, Beaulah, Laju, & Anupriya, 2012). In another study, the zone of inhibition against *E.coli*, *P.aeruginosa* and *Aspergillus flavus* was recorded 15 mm, 20 mm and 15 mm respectively (Arunkumar & Muthuselvam, 2009). In current research, the mean zone of inhibition is 15 mm for both *E.coli* and *P.aeruginosa* and 16 mm for *Aspergillus tubingensis* (Figure 4 and Figure 5).

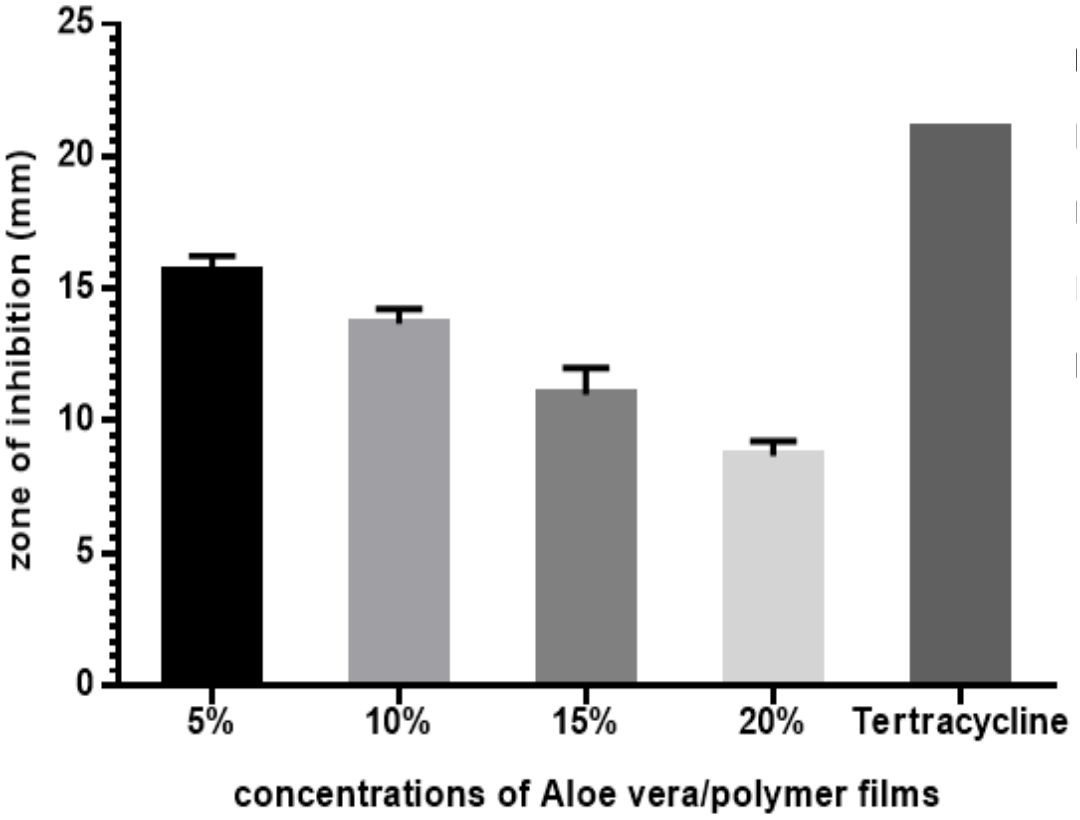
A



B


30
20
10
0
zone of inhibition (mm)
5%
10%
15%
20%
Amphotericin B
concentrations of Aloe vera/polymer films

C


25
20
15
10
5
0
zone of inhibition (mm)
5%
10%
15%
20%
Tertracycline
concentrations of Aloe vera/polymer films

D

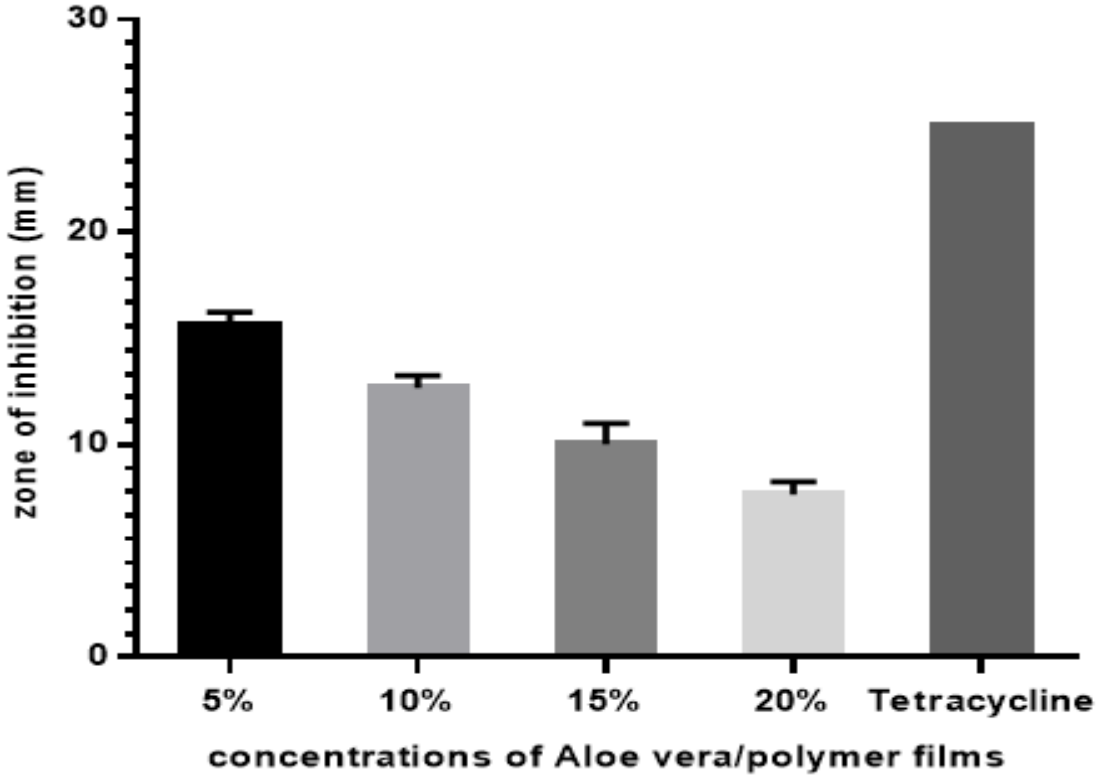


Figure 3: Graphical representation of antifungal and antibacterial activity of different concentrations of Aloe vera/polymer films. A and B shows antifungal zone of inhibition, while C and D shows antibacterial zone of inhibition. Y axis shows zones in mm while x axis shows varying concentration of Aloe vera/polymer films.

4.4 Degradation and drug release profile test results

The degradation profile of Aloe vera/PVA composite was evaluated by recording weight loss at pre-determined time points (Figure 4). The degradation profile was divided into three stages; during first 10 minutes a sudden loss of weight was observed due to the initial burst release of Aloe vera, followed by sudden increase in the weight of the films (Figure 4) because of the absorption of buffer solution by the PVA. PVA, when exposed to aqueous media, absorbs the liquid and swells, resulting in an increase in weight; later becomes solvated and starts losing mass (Kenawy,

Abdel-Hay, El-Newehy, & Wnek, 2007). However, after 30 minutes the weight loss by the films became linear. The rate of swelling of the PVA films after the initial burst decreased with the increase in the ratio of the Aloe component of the films. This was due to the fact that with the increase in the drug concentration of PVA based films, absorption of the liquid medium by the PVA decreased (Jannesari et al., 2011).

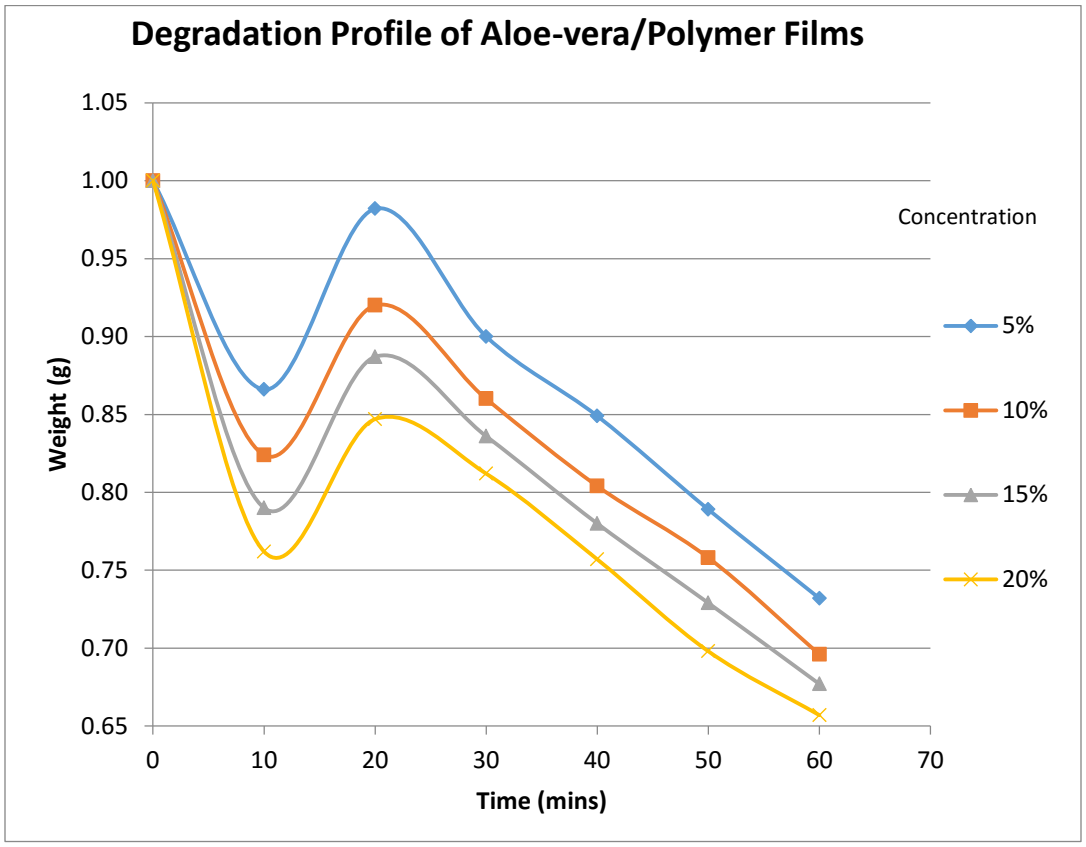


Figure 4: Degradation profile of Aloe vera/polymer films with varying concentration. Time in minutes in shown on x-axis and weight in grams is shown on y-axis.

The initial burst release followed by slow surface release of Aloe vera from the polymer based Aloe vera films was observed (Figure 5). An initial burst release of

CHAPTER 4 RESULTS AND DISCUSSION

Aloe vera from the surface of the films was detected during first 10 minutes. This may be attributed to the presence of aggregates of Aloe vera components on the film surface (verified later by SEM images of the films). The aggregates of Aloe vera over the surface of the films were observed causing the initial burst release. Later, the amount of Aloe vera released from the surface decreased because of entrapment of Aloe vera in PVA mass. During first 10 minutes Aloe vera was released only by diffusion from the surface while after 20 minutes the degradation of Aloe vera/polymer film also contributes to the release of Aloe vera (Rosenberg, Devenney, Siegel, & Dan, 2007). The release profile of all the concentrations that is, 5%, 10%, 15% and 20% showed the same behavior but with the increase in concentration from 5% to 20% greater initial burst release was observed which is due to the increased amount of Aloe vera. Moreover, increased concentration resulted in decreased drug release from the surface on the later stages because increasing the drug amount lowers the rate of diffusion of drug from the surface (Kumbar & Aminabhavi, 2003).

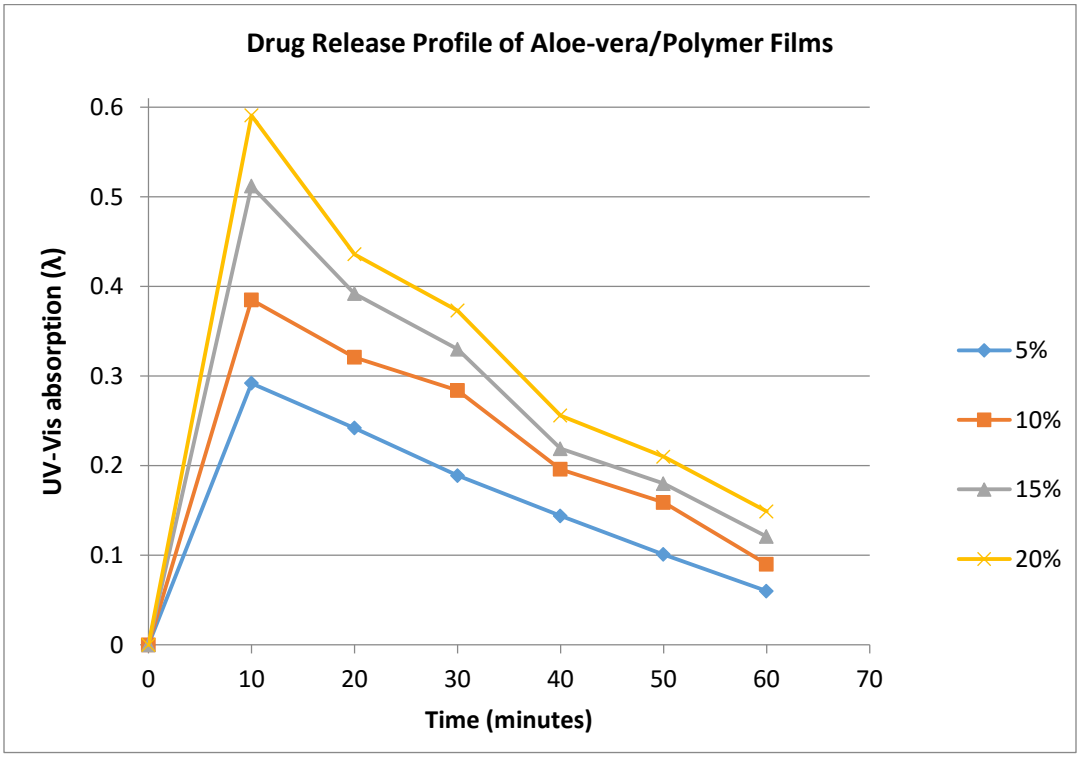


Figure 5: Drug release profile of Aloe vera/polymer films with different concentrations. X-axis shows time in minutes and y-axis shows UV-Vis absorption in λ.

The Aloe vera/polymer composite film is flexible and can easily be placed on body surfaces, hence making it an ideal candidate for wound healing devices. The initial Aloe vera release from the surface is intended to be used as an antimicrobial so as to prevent the entry and proliferation of the microbes into the wound area (S.-J. Park & Kim, 2005). Also, slow release marks the potential for an ideal microbial free environment for wound healing (S.-J. Park & Kim, 2005).

4.5 Coating of the sutures

The dry weight of the sutures before dipping into the coating solution 0.045 g and after dipping into the coating solution was increased to 0.075 g. This increase in weight demonstrated the coating of the suture with the coating material.

4.6 *In vitro* testing of coated suture and uncoated suture

The zones of inhibitions against both the bacterial strains (*E.coli and P.aeruginosa*) were evaluated with coated sutures (Figure 6). The results were compared with uncoated sutures which demonstrated no zone of inhibition, using paired t-test (Table 1)

The zone of inhibition with *E.coli* was 4.6 ± 0.577 mm (mean of three triplicates) p value = 0.0051 while with *P.aeruginosa* it was 3.16 ± 0.28 mm (mean of three triplicates), p value < 0.0028.

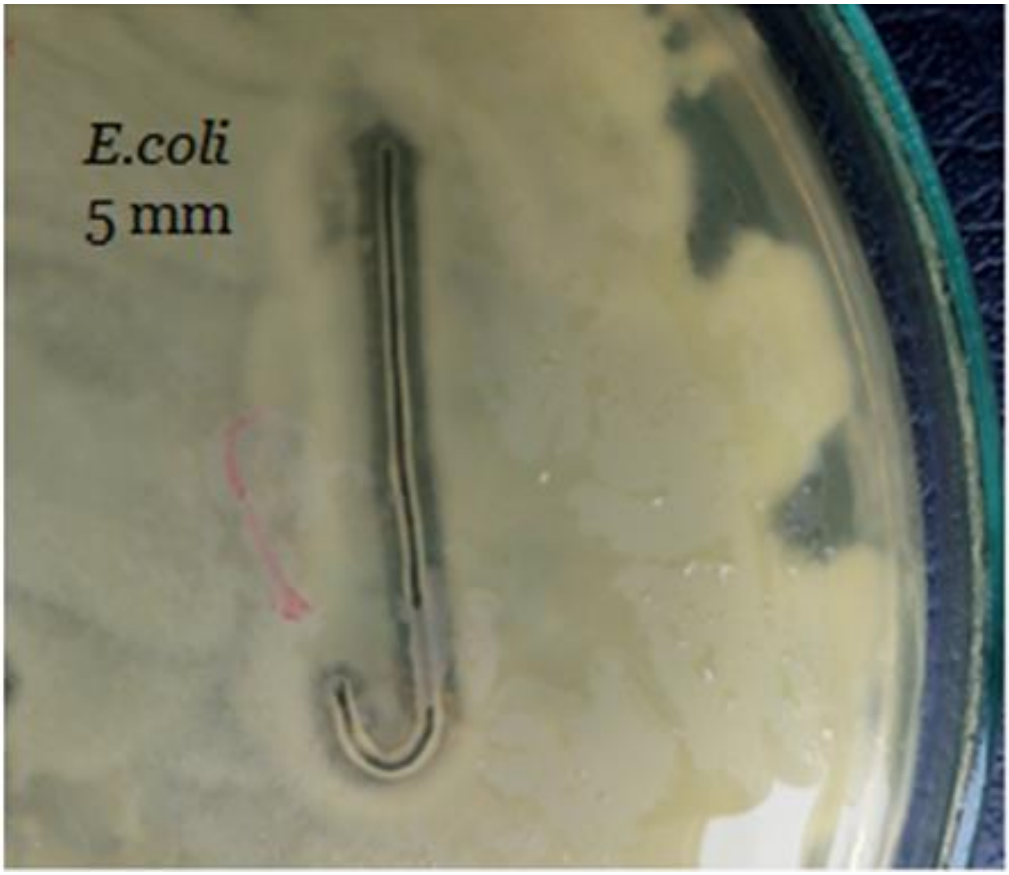


Figure 6: *In-vitro* testing of coated suture against *E.coli*

Bacteria	Zone of inhibition (mm)	
	Coated suture	Uncoated suture
E.coli	4.6 ± 0.577	0 ± 0
P.aeruginosa	3.16 ± 0.288	0 ± 0

Table 1: *In vitro* testing of coated and uncoated sutures against *E.coli* and *P.aeruginosa*

4.7 *In vivo* testing of coated sutures

The silk sutures coated with Aloe vera/polymer coatings showed significant reduction in microbial colonization by *E.coli* and *P.aeruginosa* in mice models (Table 2). The coated sutures demonstrated reduction in *E.coli* to about 97% ($p < 0.0001$) and 80% with *P.aeruginosa* ($p < 0.0001$) (Figure 7).

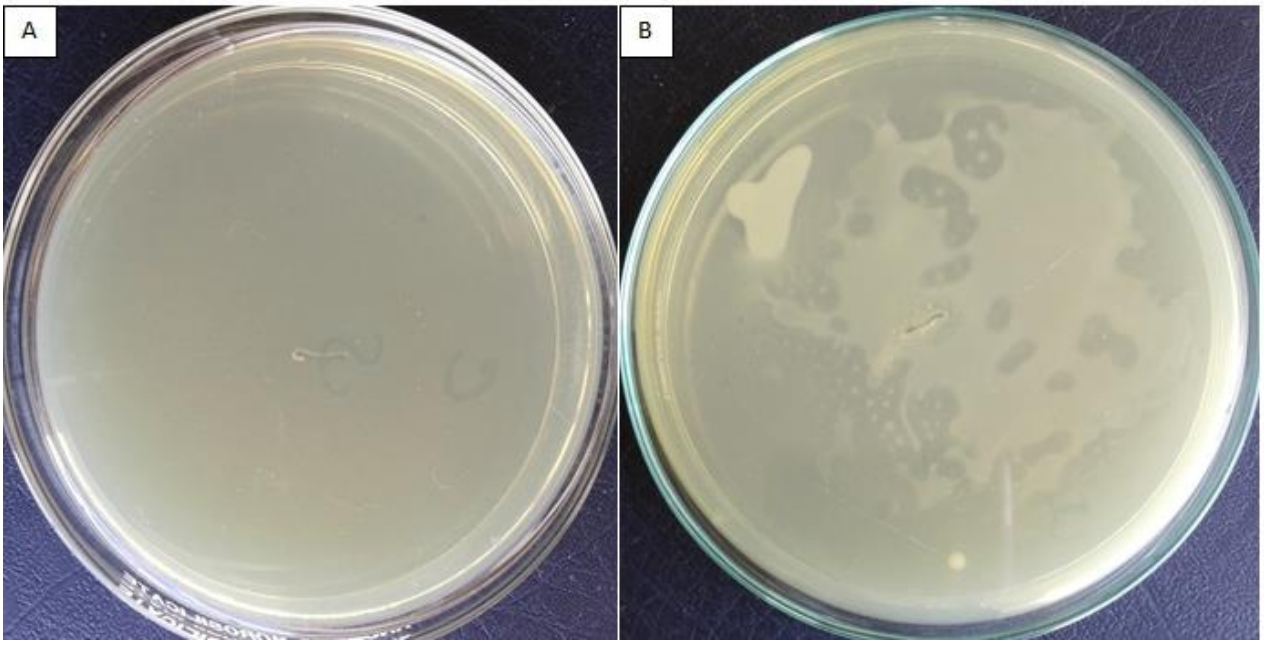


Figure 7: *In-vivo* colonization of *E.coli* with coated and uncoated sutures.(A) shows the results of *in vivo* antibacterial activity with coated suture while (B) shows the *in vivo* antibacterial results with uncoated sutures.

Bacterial Strains	Log CFU/ explanted [a]	% kill bacteria relative to inoculum introduced	P value [b]
E.coli			
With coated material	03	97	< 0.0001
Without coated material	>300	NA	
P.aeruginosa			
With coated material	11	80	< 0.0001
Without coated material	>300	NA	

Table 2: *In vivo* bacterial colonization of suture with coated material.

[a] Average of three animals

[b] Paired t test

NA = Not applicable

In this present study, silk sutures coated with Aloe vera/Polymer coating exhibited substantial zone of inhibitions against *E.coli* and *P.aeruginosa in vitro*. Coated sutures showed results against bacterial strains while no inhibition zones were observed with uncoated sutures. For *in-vivo* studies, mice models were used in which control and test sutures were used in the same animal and the incision site was inoculated with a known number of bacteria to evaluate the effectiveness of the coated sutures.

Sutures with Aloe vera/Polymer coating illustrated noticeable reduction in the growth of the *P.aeruginosa* and even greater against *E.coli.* The test results of the *in vivo* and *in-vitro* investigations suggested that sutures with Aloe vera/Polymer coating are

bactericidal. It was verified by calculating the bacterial colony count at incision site which was reduced in case of coated sutures; this shows that the Aloe-vera/PVA composite may be used as a suture coating that has the potential to prevent the spread of infections during surgical procedures.

5. CONCLUSION

The biocompatibility and bio-degradative properties of PVA have been combined with the intrinsic bactericidal properties of Aloe vera. The composition was screened for antimicrobial activity against bacterial and fungal strains that is *E.coli, P.aeruginosa, Aspergillus flavus* and *Aspergillus tubingensis* respectively. The polymeric films with lowest concentration (5%) of Aloe vera illustrated the best results with regards to antimicrobial activity against all the strains. Commercially available sutures were coated with Aloe vera/PVA solution and tested for antimicrobial activity *in-vitro* and *in vivo* systems. These coated sutures illustrated a potential for antibacterial/antifungal coatings in commercial surgical sutures that can play a role in preventing infections at surgical sites.

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