Research Article

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EVALUATING THE NATURAL FIBRE REINFORCED POLYMER BIOCOMPOSITE FOR THE DEVELOPMENT OF NOVEL WOUND DRESSING MATERIALS

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ABSTRACT

Natural fibre reinforced polymer biocomposite as 2D laminate wound dressing material was developed and evaluated for its wound healing abilities. Two fibres, thuthi and banana were selected to construct a hybrid composite fibre (HCF). Both thuthi and HCF were reinforced with a polymer, poly vinyl alcohol (PVA) to develop a 2D laminate film material. Developed material was evaluated for its antibacterial activity and wound healing ability using a standard in vitro wound scratch assay method. Antibacterial activity showed potential results by exhibiting inhibitory zones against two bacterial wound causing pathogens, *Escherichia coli* and *Staphylococcus aureus*. The developed composite also showed promising wound healing abilities when assayed under in vitro conditions. The method implemented in this research to develop biocomposite shall be used for other fibres to optimize and determine the enhanced antibacterial actions and wound healing abilities. In future the natural fibre reinforced polymer biocomposite shall also be used in different forms and structures to meet the needs in the field of pharmaceutical science and medical textiles.

Keywords: Plant fibre, Reinforced, 2D laminate, Biocomposite, wound dressing film

INTRODUCTION

Natural fibres and its applications are growing in many sectors such as automobiles, constructions, furniture, packing and cosmetics, medicine and for other biopolymers and fine chemicals[1]. This is mainly due to their advantages compared to synthetic fibres, *i.e.* low cost, low weight, less damage to processing equipment, improved surface finish of moulded parts composite, good relative mechanical properties, abundant and renewable resources[2]. Natural fibres are used in medical textiles since it possess a high strength to weight ratio, high fracture toughness, non-corrosive nature, renewability and sustainability[3].

Some of the natural fibres are bamboo, kenaf, sisal, hemp, jute, silk and cotton. Bamboo significantly consists of cellulose fibre embedded in a lignin matrix; hence they are traditionally used as a material for the manufacture of tools. Bamboo naturally has high strength to weight ratio[4]. Other fibre called Kenaf is one of the natural (plant) fibre used as reinforcement in Polymer Matrix Composites (PMCs); it is well known for its rich cellulose fibre content[5]. An important hard fibre which is extracted from the leaves of plant (*Agave sisalana*) is termed as sisal fibre. Due to its hard and high tensile structure it is widely used in the construction industries[6]. It was reported that these plant-based natural fibres have been frequently used in the manufacturing of biocomposites. Biocomposites are largely utilized in biomedical applications such as drug and gene delivery, tissue engineering, orthopedics, and cosmetic orthodontics[7]. Biocomposites can be fabricated by combining biofibres such as kenaf, industrial hemp, flax, jute, pineapple fibre, sisal, wood, and various grasses[8] with bio-binders, commonly known as biopolymers[9]. Polylactic acid (PLA) and polyglycolic acid (PGA) are the two major polymers. These bio binders can degrade inside the body at a rate that can be controlled; their degradation products are nontoxic, biocompatible, and easily metabolized[10]. Bio-binders find many applications in a number of fields such as drug delivery system, wound healing, food containers and agricultural films, waste bags, soil retention sheeting, filtration, hygiene and protective clothing, and automobile industries[11]. Biocomposites are widely applied in the field of biomedical applications[12]. Alternatively, biocomposites are also used in other applications which are reported in the form of a natural fibre or in the form of plant fibre component. The primary reason for the development of biocomposites from natural fibre is, they are highly flexible, distribution of the reinforcing phases in the composites and as a wide range of mechanical and biological properties[13]. They may be engineered into the development of the next generation of materials, products, and processes[14].

In the present research, two different plant fibre was reinforced with a bio-binder polymer called poly vinyl alcohol (PVA) to develop a 2D laminate biocomposite film. The developed biocomposite films were evaluated as wound dressing material under *invitro* methods. Thuthi plant fibre and banana stalk fibre selected in this research were based on the properties and applications of biocomposite in medical fibres. The biomedical natural fibre reinforced polymer biocomposite was evaluated for its antibacterial activity and biocompatibility using standard methods.

MATERIALS AND METHODS

The present research work was carried out in PG and Research Department of Costume and Fashion Technology, Dr. NGP College of Arts and Science, Coimbatore, India. The entire research work was performed from January 2017 to April 2017.

Collection and fibre extraction[15]

Thuthi (*Abutilon indicum*) and banana were procured commercially and the fibres were extracted using fibre extractor equipment by the method called scraping. Scraping was carried out in two stages called linear scrapping and rotational scrapping. Long fibre was extruded by the means of scrapping method. The fibre length ranging from 1 to 2 m was chopped in a developed machine to small lengths according to the requirements of the enzymatic treatment (5 to10mm). The quality and contamination free status of the selected fibre was determined using standard protocols. The defined size and structure of the fibres were observed under hand microscope before pretreament. During the microscopic analysis, no other plant fibre was observed with either thuthi or banana fibers.

Enzymatic treatment of fibre[16]

Biopectinase K is the enzyme of choice was selected based on the presence of plant components like cellulose, hemicellulose, pectin and lignin. The treatment time and enzyme concentration were selected based on fibre weight. Fibre to bath volume ratio was set to 1:40; temperature was set at 45C and pH at 4.5. The enzyme treatment time varies from 1 to 5 h and the enzyme concentration from 1% to 5%. After enzyme treatment the plant fibres were screened based on their tensile strength. Selected fibres of thuthi and banana were further processed to obtain a hybrid composite structure. The hybrid composite fibre (HCF) was prepared by adding equal weight of thuthi and banana fibres at a ratio of 1:1.

Development of wound dressing 2D Laminate Biocomposite films

Enzyme treated plant fibre (thuthi fibre and hybrid composite fibre) was dried at room temperature and sterilized at autoclave conditions. Thuthi fibres and HCF was used separately to develop a 2D laminate biocomposite films. The biocomposite film was developed with bio binder called poly vinyl alcohol. To enhance the laminate film formation, a bridging agent called glutaraldehyde (GA) was used. GA enhances mechanical properties and biostability of natural fibres.

About 10g (1X), 20g (2X) and 30g (3X) of Thuthi fibre and HCF were weighed separately. In a sterile polycarbonate petridish, 20ml of poly vinyl alcohol was added. GA was used in a concentration of 0.25% as a bridging agent. The pre-weighed plant fibres were added slowly over the surface of PVA and GA mixtures. Care was taken to ensure whether the fibres were spreaded evenly on the surface of PVA and GA mixture. Hence, the plates were kept in an orbital shaker (80rpm) to disperse the fibres evenly. All the plates with added fibres were kept in an oven under the temperature set at 50C. All the plates were dried till the mixtures gets evaporated to form a final thin laminate film on the bottom surface of polycarbonate petridish. The developed 2D laminate composite films were carefully removed from the petridish surface and stored at 4C for further analysis.

SEM analysis of wound dressing 2D Laminate Biocomposite films

Developed 2D laminate biocomposite wound dressing films were observed using Scanning electron microscopy. SEM evaluation was used to know the uniformity dispersion of thuthi and HCF on the developed films. All the test materials were prepared for SEM using a suitable accelerating voltage (10 KV), vacuum (below 5 Pa) and magnification (X 3500). Metal coating was used as the conducting material to analyze the sample.

***In Vitro* Antibacterial Test (EN ISO 20645 test method)[17]**

The antibacterial performance of the developed wound dressing 2D laminate was evaluated using an *in vitro* antibacterial test. About 100µL of *Escherichia coli* and *Staphylococcus aureus* in Nutrient broth suspension with a concentration of 10^5–10^7 CFU/mL was swabbed on a Nutrient agar plate separately. The 2D laminate film was cut as per standard size (2.5cm in diameter) and placed over the inoculated Nutrient Agar plates. All the plates were kept for incubation at 37ºC for 24h. At the end of incubation, zone of incubation formed around the film was measured in millimeter and recorded.

Wound healing assay of 2D laminate composite films: *In vitro* Wound Scratch Assay[18]

L929 mouse fibroblast cells were grown in 24 well plates at a density of $1X10^5$ cells/ml and cultured until ~ 80% confluency. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper as per the method described by Liang et al. (2007). Cells were thoroughly rinsed with 1 X PBS to remove cellular debris and treated with 2D laminate wound dressing films dissolved in PVA at a ratio of 1:1 (25 µl). Cell proliferation was monitored at different time points: 0, 24, and 48 h and images of the migrated cells were taken at all different time points using digital camera (Nikon, Tokyo, Japan) connected to the inverted phase contrast microscope (Radical instruments, India). Extent of wound healing was determined by the distance traversed by cells migrating into the denuded area.

RESULTS

Microscopic observations of the enzyme treated plant fibres

Microscopic observations of thuthi and banana fibres were carried out two different magnifications under polarized light using a stereo-zoom microscope. The width of the fibres before and after enzyme wash was observed and measured. The differences in fibre surface observations between before and after wash enzyme treatment were observed for thuthi and banana fibre respectively. Difference in pretreated fibres of thuthi and banana were mainly due to the action of biopectinase K. The enzyme played a crucial role in removing the undesired compounds like pectin and hemicellulose from the fibre surface.

Physical observations of developed 2D Laminate Biocomposite films

The plant fibre reinforced polymer biocomposite are categorised under the type called 2D laminate composite. Three laminate films developed using 1X, 2X and 3X fibre volumes of thuthi (Figure 1 and 1a) and hybrid composites (thuthi + banana – Figure 2 and 2a) were observed physically to differentiate among each film. Since no difference among the three laminate films were observed through physical observations, all the developed films were further subjected to SEM analysis.

SEM analysis of wound dressing 2D Laminate Biocomposite films

SEM images of the developed biocomposite films revealed the fibre structure, approximate size and difference in the dispersion of fibres throughout the films depending on the concentration of fibres. The difference in fibre volumes among the three strengths used for the development of composites 1X, 2X and 3X concentrations were noted clearly during SEM analysis. The difference in fibre volume in each laminate composite film was compared with their respective antibacterial assay after evaluating against the test pathogens.

In Vitro Antibacterial Test

Antibacterial activity of the developed biocomposite was challenged against two significant pathogens, *Escherichia coli* and *Staphylococcus aureus.* Among the three different films developed using thuthi fibre and hybrid composite fibre, the laminate films with 3X fibre volumes were found to produce more inhibitory zones indicating the potential antibacterial actions against the challenge organisms. In Figure 3 and Figure 4, the inhibitory zones thus produced were presented to differentiate the antibacterial activity of three different films. In Table-1, the inhibitory zone measured in millimetre was presented to evaluate and select the appropriate films for wound dressing applications.

Self-wound healing scratch assay: *In vitro* Wound Scratch Assay

In vitro wound healing assays have commonly been applied to measure cell migration, cell proliferation and wound closure in response to stimulation with specific agents. In this study, the developed 2D laminate biocomposite films were evaluated for its biocompatibility and ability to improve wound healing by acting directly on L929 mouse fibroblast cells.

After creating a scratch on L929 mouse fibroblast cell lines, the cell migration, cell proliferation and wound closure was measured for a known concentration (100µg) of developed laminate films at three different time periods (0^{th} hour, 12^{th} hour and 24^{th} hour). Figure-5 corresponding to self-wound healing ability of the laminate film showed that, at 0^{th} hour, no cell migration and proliferation was observed for the known concentrate (100µg) including control (Distilled water). At 12^{th} hour, the samples showed positive cell migration and cell proliferation when compared to the control sample. After 24hours, more cell proliferation was evident and thus indicating the wound healing ability.

As there was similar wound healing abilities were noted for many plant fibre constituents from the literature survey on L929 mouse fibroblast cell lines, it was proved that the developed biocomposite would replace all other synthetic wound dressing materials with good biocompatibility in human tissues.

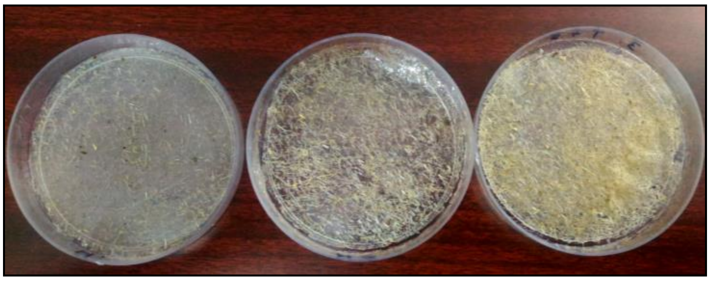


Figure 1a: Physical observations of developed laminate film (1X, 2X and 3X thuthi fibre)

Figure 1b: 2D laminate biocomposite film developed with thuthi fibre (3X)

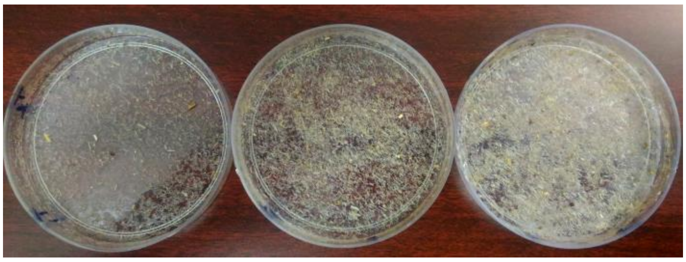


Figure 2a: Physical observations of developed laminate film (1X, 2X and 3X thuthi fibre)

Figure 2b: 2D laminate biocomposite film developed with thuthi fibre (3X)

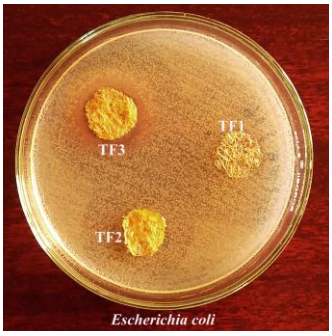


Figure 3a: Thuthi film against *E.coli*
TF1 (1X), TF2 (2X), TF3 (3X)

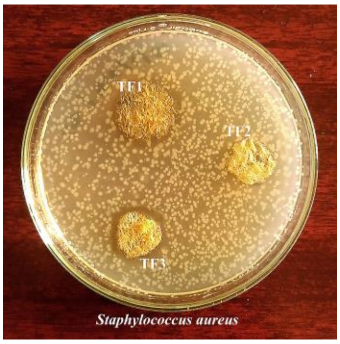


Figure 3b: Thuthi film against *S. aureus*

Figure 4a: HCF film against *E.coli*
HCF1 (1X), HCF2 (2X), HCF3 (3X)

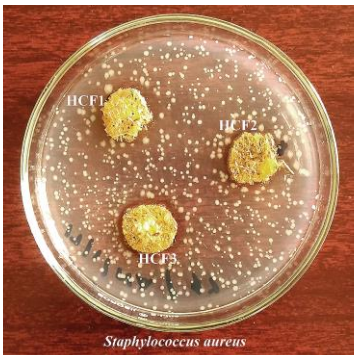
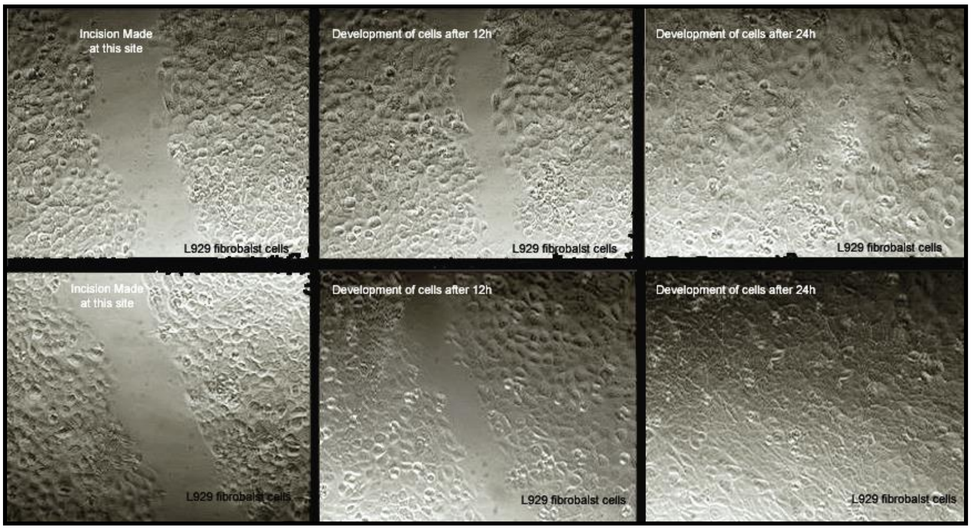


Figure 4b: HCF film against *S. aureus*



0h 12h 24h

Figure 5: Self-wound healing scratch assay: *In vitro* Wound Scratch Assay
A: Control (Distilled water) [Top three pictures]
B: Developed 2D laminate polymer composite samples [Bottom three pictures]

Table 1: Antibacterial activity of the developed biocomposite against two wound pathogens

S. No.	Fibre	Fibre Strength	Antibacterial activity (Zone of inhibition)	
			Escherichia coli	***Staphylococcus aureus***
1	**Thuthi**	**1X** (TF1)	0	0
		2X (TF2)	0	0
		3X (TF3)	35mm	31mm
2	**Hybrid composite**	**1X** (HCF1)	0	0
		2X (HCF2)	0	0
		3X (HCF3)	39mm	33mm

DISCUSSION

The use of natural fibre as reinforcement in polymer matrix focused the attention towards environmental awareness among all over the world[19]. It has been a proven alternative to synthetic fibre reinforced polymer composites in many applications[20]. Recently, banana fibre reinforced composites are coming into in interest due to the innovative application of banana fibre in different industries like medical, textile woven, textile nonwoven, automobile and construction[21]. A hybrid composite is a combination of two or more different types of fibre in which one type of fibre balance the deficiency of another fibre[22]. Many natural fibre composite products are developed and marketed; and very few other natural fibre composites are under development with the aid of different textile technologies[23].

With this conceptual detail of natural fibres, in the present research thuthi plant fibre was extracted and made into a hybrid composite fibre by reinforcing with banana fibre. Thuthi fibre as stand-alone fibre and hybrid composite fibre (thuthi and banana) was used for the development of reinforced polymer composites. As per literature survey, thuthi plant fibre is used for the first time in the current research for the development of biocomposite and hybrid composites. Accoriding to Farideh Namvar et al biocomposite are made into two major types; either as 2D laminate or as 3D laminate. Examples for 2D laminate are UD laminate and tape laminate; and knitted, woven, stitched composites are the examples of 3d laminate. In the present research one such 2D laminate biocomposite was developed and as a novel approach, the developed biocomposite was evaluated for its ability to use as a wound dressing film laminates.

Both thuthi and banana fibres after treating with biopectinase K enzymes became bright and soft. Increase in softness of the fibres was mainly due to the softener action of the biopectinase K. Enzyme also plays a crucial role in removing some of the undesirable compounds like hemicellulose and pectin[24]. According to Indrani Sarma and Deka[25] pectinase have the ability to remove heavily coated, non-cellulosic gummy material from the cellulosic part of plant fibres. In this study similar action of biopectinase K on the plant fibres were observed through optical microscopic images and scanning electron microscopic images.

Antibacterial activity of the biocomposite showed promising results in the present research. The obtained results emphasize the role of developed laminate as a potential wound dressing materials. The reason behind the antibacterial properties of the laminate film was illustrated with the antibacterial mode of action of the plant fibres from the literature survey. Appendino et al[26] explained that the plant fibre contain numerous biologically active compounds, many of which have been shown to have antibacterial properties. Turner et al[27] observed these compounds very earlier from different fibres. These compounds are terpenes, phenols, flavonoids, pigments, ketones, enzymes etc. Recently, the total number of natural compounds identified in the plant fibre was greater than 500[28]. Radwan et al. [29] stated that the complex macrocomposition of plant fibre with these natural compounds have the potential to exhibit antibacterial activity.

Due to antibacterial potential of natural compounds, the plant fibres were incorporated as antibacterial filler in the polymer composites. These fillers prevent the bacterial attachment on the surface of any biomedical materials like wound dressings and implants or stents. The natural compounds of plant fibres like cannabinoids and alkaloids have the potential to be used as a drug to treat bacterial infection or may be used in conjunction with antibiotics to enhance their activity.

Polymers used for the development of laminate in the research also reported to have a synergistic antibacterial effect with the plant fibre. A large number of polymers such as polyethylene (PE), polyurethane (PU), polytetrafluoroethylene (PTFE), polyacetal (PA), polymethylmethacrylate (PMMA), polyethylene terepthalate (PET), silicone rubber (SR), polysulfone (PS), polyetheretherketone (PEEK), poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and poly vinyl alcohol are used in various biomedical applications due to their antibacterial potentials[13].

Thuthi and banana fibres used in this research also reported to contain many of these natural compounds natively. Even though there was no much information was available for thuthi fibres, banana fibre and its antibacterial compounds were described elsewhere by many researchers. Due to the presence of many natural compounds, the fibres were used in many biomedical applications. One such application as wound dressing material was used in this research. Many similar research works were carried out to emphasize the significance of fibre reinforced polymer composites in biomedical applications.

Ramakrishna et al.[13] used polymer composites in the form of implants and medical devices. The researchers stated that the antibacterial property may increase the functionality of polymer composites, especially in wound dressing materials. These skin dressings should prevent loss of fluids, electrolytes, and other biomolecules from the wound and obstruct bacterial entry, but should also be permeable enough to allow the passage of discharges from pores or cuts.

It was interesting to state that the plant fibres like hemp, banana and cotton can be used in conjunction with suitable materials to meet these requirements. These fibres are frequently used in wound dressings due to their excellent barrier properties and oxygen permeability. Apart from their antibacterial properties, porous physical structure, air-permeability and absorbency capabilities of the plant fibre was found to have more advantageous features to use as wound dressing materials.

CONCLUSION AND FUTURE PERSPECTIVES

Thuthi and banana fibre was reinforced separately and also as a hybrid composite with a polymer to form biocomposite. The developed 2D laminate composite was evaluated to use as wound dressing materials by determining its antibacterial action against significant pathogens and biocompatible properties by in vitro scratch assay method. The 2D laminate composite showed promising antibacterial action against the pathogens. The composite were also proved to be biocompatible material for human applications. The method implemented to develop biocomposite using thuthi and banana fibre shall be used in future for other fibres to optimize and determine the enhanced antibacterial actions against wound causing pathogens. In future the plant fibre selected in this research shall also be used in different forms to fulfill the needs of biomedical applications or medical textiles.

Plant fibre incorporated nanofibrous poly urethane membrane developed by electrospinning, could be employed as wound dressings. Plant fibres shall also be incorporated with asymmetric chitosan or alginate polymers for the development of advanced wound dressing membrane with the antibacterial capacity to prevent infection in diabetes patients.

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