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Fabrication of a Novel Aloe Vera (AV) and Polyvinyl Alcohol (PVA) as Dressing for Facial Abrasion

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Aim: To fabricate Aloe vera in the form of a film /sheet with the help of Poly Vinyl Alcohol

Materials and Methods:

Objectives: The fabrication of Aloe vera in the form a sheet/film with the help of Poly Vinyl Alcohol was done. The Percentage compositions of Aloe vera tested were 6%,12% 18% along with standard PVA 9%. Physical Characterization and Biocompatibility studies were performed.

Outcome Variables: Fabricated films are non -toxic and biocompatible to patients

Result: The three different compositions of Aloe vera (AV) with Poly vinyl alcohol (PVA) 6%,12%,18% has shown good results and are safe for therapeutical application. The Studies performed after fabrication showed good tensile strength, porosity, non-cytotoxic ,good flexibility .

Discussion: The Aloe-vera with polyvinyl alcohol had shown good results and are safe for therapeutical application and hence this was proved by characterization studies.

Conclusion: The Aloe vera was made in the form of a film which was bio-compatible, non-toxic and no adverse chemical interactions between Aloe vera and Poly vinyl alcohol materials was observed.

I. INTRODUCTION

In the maxillofacial trauma, the soft tissue injuries with or without involvement of facial bone are the most common. Break down and discontinuation of epithelium results in soft tissue injuries. There are three types of injuries mainly physical, thermal and chemical injuries. Wound and related injuries remain a major cause of death and disability.(Barreto et al., 2014) proposed that the wound healing is acomplex, highly regulated process that includes cellular, molecular, biochemical, and physiologicalevents that permit living organisms to repair accidental lesions. This process includes 3 overlappingphases: inflammation, proliferation and tissue formation, and tissue remodeling. These events areinitiated at the time of physical injury and continue throughout the healing process.

Wound exudate provides a warm and moist environment in which bacteria can arise and proliferate, thus, increasing the bioburden of the wound. Inoculation of such wounds can lead to bacterial colonization, biofilm formation and later on to infection. It has been reported that only 3.6% of surgical wounds exposed for less than 30 min are infected, whereas 16.4% of the wounds exposed for longer than 5 hours develop infection.

The objective of wound management is to heal wounds in the shortest amount of time with minimal pain, discomfort, and scarring and prevent infection. Thus, improving treatment for wound healing and tissue repair will improve the quality of life of patients with wounds as well

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as reduce the overall cost of wound-related health care. There are many numbers of dressing materials which are natural, semi synthetic or herbal that are commercially available; but healing time is still delayed.

In herbal form only dressing medicaments are available that uses 3% aloe vera and showed good anti-inflammatory properties and re-epithelization of wound. The properties were explained in fig 1.

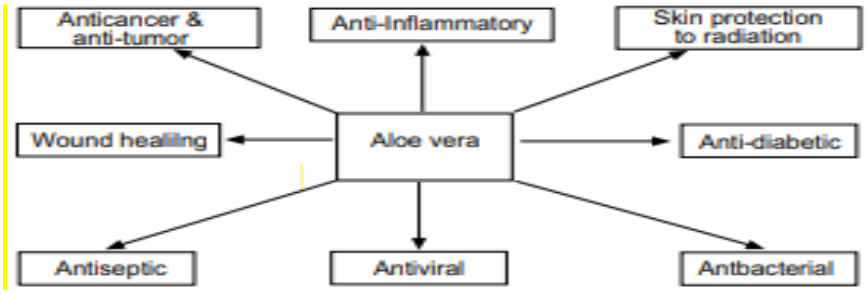

Fig. 1: Medical Properties of Aloe vera (Ahmed and Hussain, 2013)

Most of the existing literature says that aloe vera promotes wound healing in cases of burns and frost bite. As per literature various studies have shown that the aloe vera has excellent anti-inflammatory properties and is fabricated in the forms of gels with additive dressing materials, ointments for cosmetic purpose. Although there are number of commercially available synthetic, semisynthetic, natural, alloplastic dressing materials, they take a longer wound healing time. Over a period of time as the wound is healing these dressing materials causes discoloration of the skin some of these medicaments are bovine in origin and hence patient compliance towards it is low. Hence there is a need for novel herbal dressing material with good anti-inflammatory, anti-microbial, anti-scarring material that will also provide good patient acceptance in the view of its natural origin.

Polyvinyl Alcohol(PVA) is a water soluble and had multi-purpose properties and used in cosmetics and personal care products polyvinyl alcohol functions as a gel based thickener ,film forming agent and advantages of binding capacity.Its ability to dry into a thin film on the surface of skin that can be used in cosmetic materials .PVA mixed with other ingredients protect the skin .

However aloevera and pva were combined to form a flim which will give good healing index .so the blending of aloevera with PVA with different formulation were made.

II. METHODS AND METHODOLOGY

- **Objectives:** To fabricate Aloe vera in the form of films/sheets.

III. MATERIALS

In First experiment, Aloe vera was washed under running water and the yellow extract was allowed to flow till the green colour gel run at the corner of the leaf was seen and both the ends were cut by using knife and upper layer were peeled of, gel was collectedwas thoroughly blended by using electric blender.fig 2.

Fig. 2: Aloe vera Leaf ,Aloe vera gel in beaker

Fig. 3: Aloe Vera Gel

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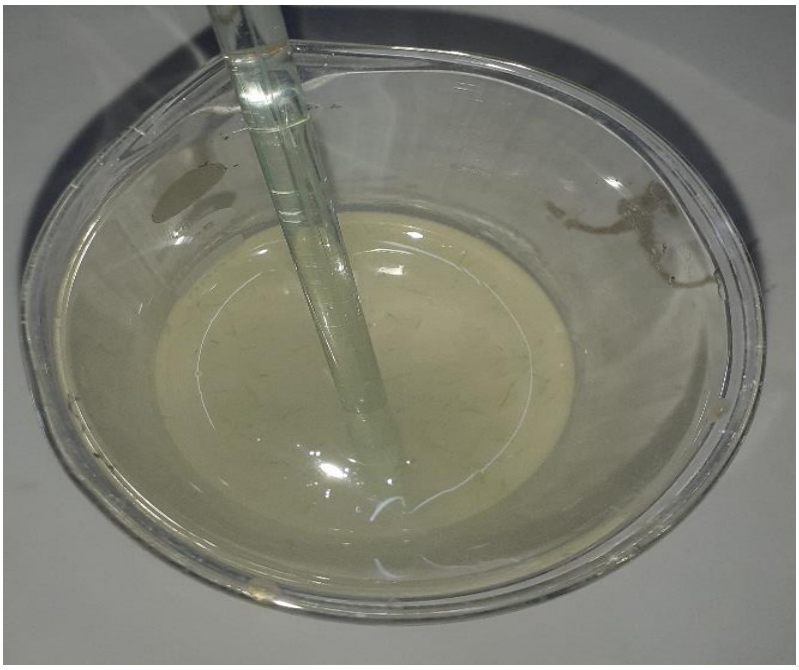

Fig. 4: Aloe Vera Mixture without PVA

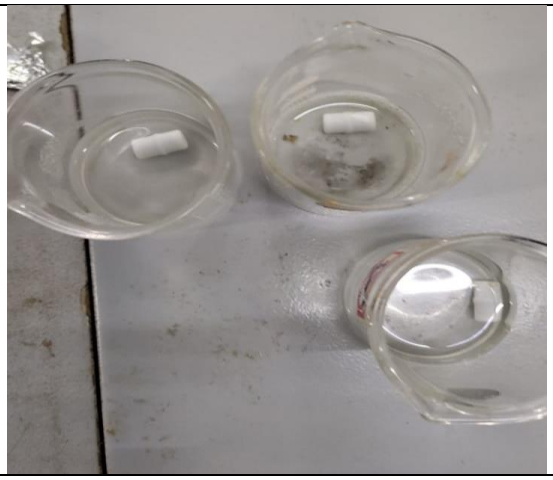

Fig. 5: PVA Solutions

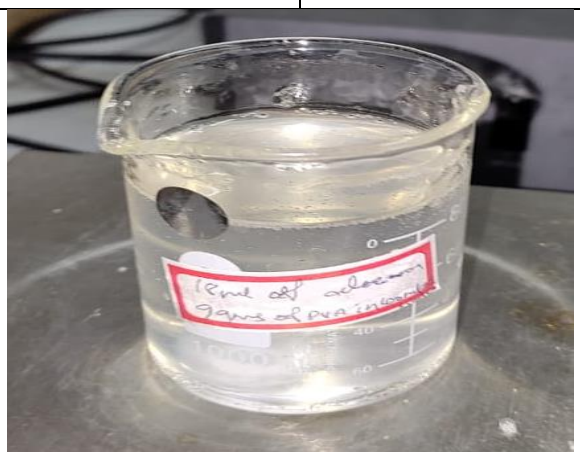

Fig. 6: PVA with Aloe Vera

Fig. 7: PVA with Aloe Vera Sample-1(Successful trial

Fig. 8: PVA with Aloe Vera Sample-2(Successful trial

Fig. 9: PVA with Aloe Vera Sample -3(Successful trial

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A. PREPARATION OF ALOE VERA GEL

Aloe vera leaf was obtained, washed thoroughly under running water, yellow extract which is toxic was removed. Then lateral aspects of leaf were cut, upper. layer was removed and gel was extracted. The extracted gel was thoroughly blended by using electrical blender then filtered and stored in refrigerator for 10 min and used.

B. PREPARATION OF PVA solution (9%)

The poly vinyl alcohol of 9 grams weighed in the weighing machine by using butter paper and 100ml of distilled water was taken in measuring cylinder. Weighed Poly vinyl alcohol of 9 grams was transferred in to the beaker and 90ml of distilled water was made up to 100ml and distilled water was added and stirred. Next, the mixture was placed in magnetic stirrer by using beads in the beaker at 80 °cand 2100 rpm for 2 hours. It was allowed to cool for 15mins. (Fig 5)

C. PREPARATION of 6% ALOE VERA,12% ALOE VERA,18% ALOEVERA

Once mixture cooled, three beakers of 100ml was taken and 1st beaker consists the aloe vera of 60 ml S1 (w/v) 6% (0.6),2ndS2 12% 120ml/l (1.2) of aloe vera, S3 18% (1.8(w/v) 180ml/of aloe vera was poured, then made up to 100ml.The mixture was stirred once by using magnetic stirrer for 2 hours under 80 °c to get homogenous mixture. Then the mixture was cooled for 15 mins. Petri plates of 7cm was chosen, then the mixture was poured in three different petri plates named as sample 1(6%), sample 2(12%) and sample 3(18%), placed in hot air oven for 3 days at 70 degrees centigrade. The formed Aloe vera films were peeled off, and stored in self- sealing pouches. Fig 7 Fig 8 Fig 9.

IV. RESULTS

Fabrication of aloe vera loaded PVA films was carried out and the following characterization studies was performed. The physical charateistic studies were performed ,they are Fourier-transform infrared spectroscopy,Scanning Electron Microscopy,Tensile strength% ,Elongation Optical Profilometry,with Cytotoxic Studies (MTT- ASSAY)

V. DISCUSSION

One of the oldest medicinal plants ,200 or more different biologically active properties had attracted researchers, contains acemannan which is the key functional component had long chain polymer in its structure with linear d-mannopyranosyl units along with immune modulation, antibacterial, antifungal and antitumor properties.

A. Fourier-transform infrared spectroscopy Studies

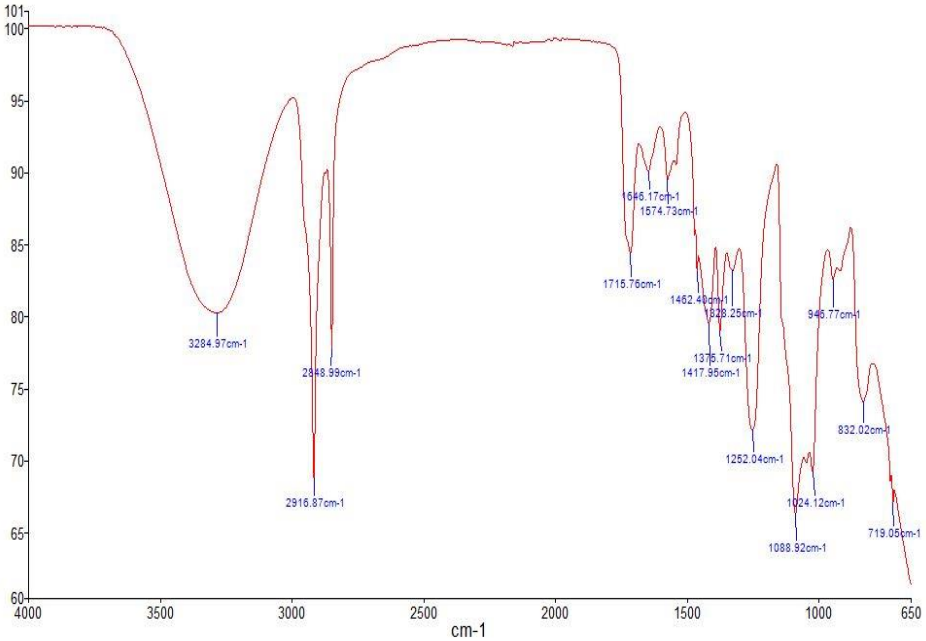

Fig. 10: (ATR-FTIR for PVA)

In the spectrum of pure PVA broad band observed 3284 cm^{-1} was ascribed to O-H and N-H stretching vibration from the intermolecular and intra molecular hydrogen bonds. $=CH_2$ bonds were found at 2917cm^{-1}, described as stretching of CH_2 (Abdelghani, Menazea et al, 2019) (Fan, L.Yang, H, Yang et.al 2016). -C=O (carbonyl group) stretching was found at 1690cm^{-1}.-C-H band observed due to bending at 1425cm^{-1}. A band at 1324 cm^{-1} observed due to deformative bonding .1081 $cm^{-1}$was due to stretching of acetyl group C-O-.839cm $^{-1}$band was observed due to stretching vibration.

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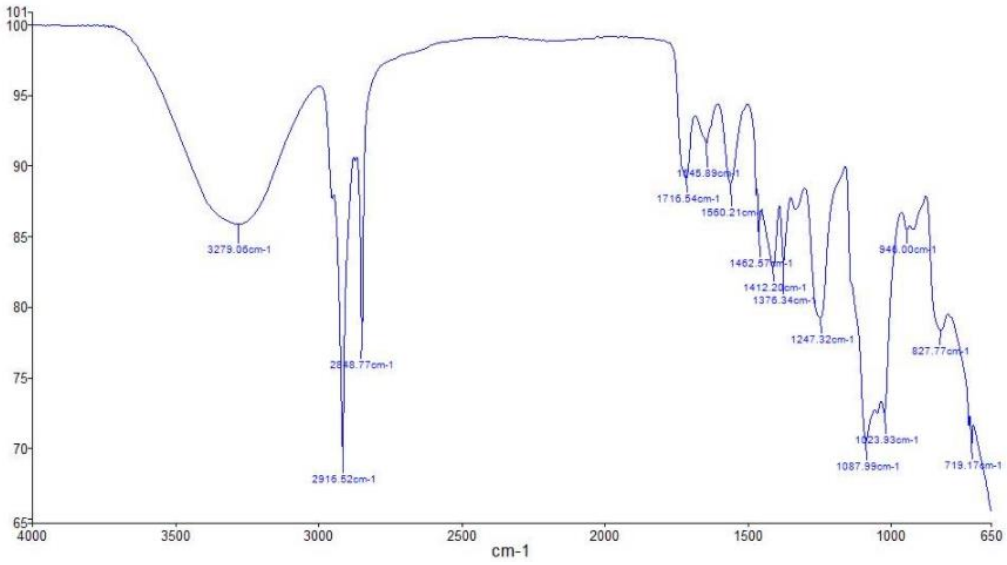

Fig. 11: (PVA with Aloe Vera)

In this spectrum of PVA and Aloe Vera broad band observed at 3279 cm^{-1} was described to be -OH bond due to stretching at 2916 cm^{-1} band was observed due to -CH_2stretching.1645 cm^{-1}-C=O carbonyl stretching ((Abdelghani, Menazea et al, 2019) (Fan, L.Yang, H, Yang et.al 2016). The band observed at 1462 cm^{-1} was due to -C-H bending .1376 cm^{-1}band was due to deformative vibration in the bond -C-H-. The band at 1087$cm^{-1}$was observed due to stretching of acetyl group -C-O-. The band at 827 $cm^{-1}$was observed due to stretching and vibrations in -C-C- (Abdelghani, Menazea et al, 2019) (Fan, LYang, H, Yang et.al 2016).

The characteristic peaks which were observed in PVA were also present in the mixture of PVA + Aloe vera which confirms absence of any chemical incompatibility or reaction between the polymer and the plant extract. The change in characteristic peaks in the blend of PVA+ aloe vera if present are only due to the physical interaction which can be seen from very little shifts of characteristic peaks.

B. Scanning Electron Microscopy (SEM)

The morphological characterization of the Aloe vera-containing PVA films as well as the PVA were determined through Scanning Electron Microscopy (SEM)(Jegina, S., Kukle, S. and Gravitis, J., 2018, December).From the images it was observed that the surface morphology of the PVA films was smooth, homogeneous with almost no straps. Whereas from images of PVA+AVfilms it was observed that the surface morphology had roughness which may be due to the addition of aloe vera that made the topography of the film little rough. Further, in both the right side images, we can observe pores although the pores are closed which may open up after the application of the films , due to swelling of the same.

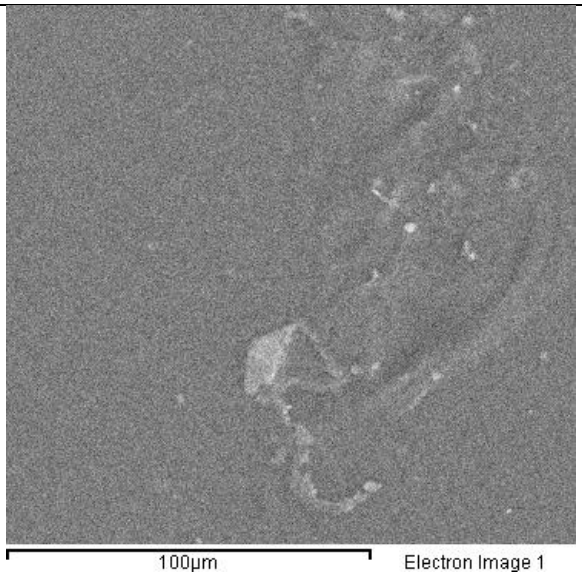

Fig. 12: (PVA Films)

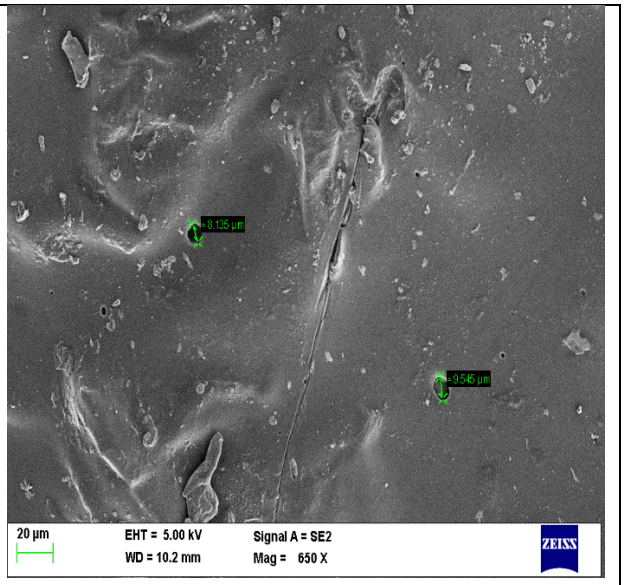

Fig. 13: (PVA Flim with Pores)

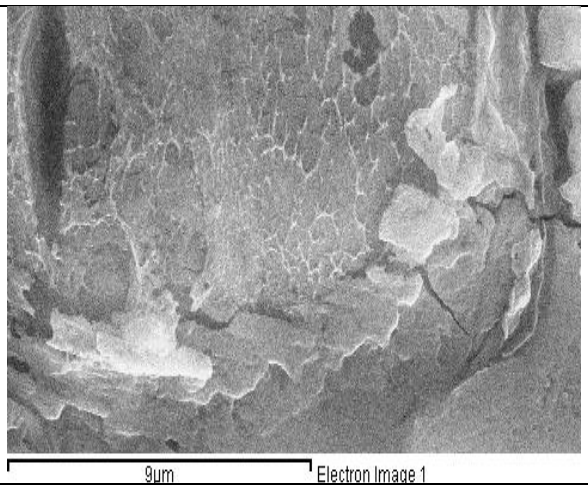

Fig. 14: (PVA with Aloe Vera)

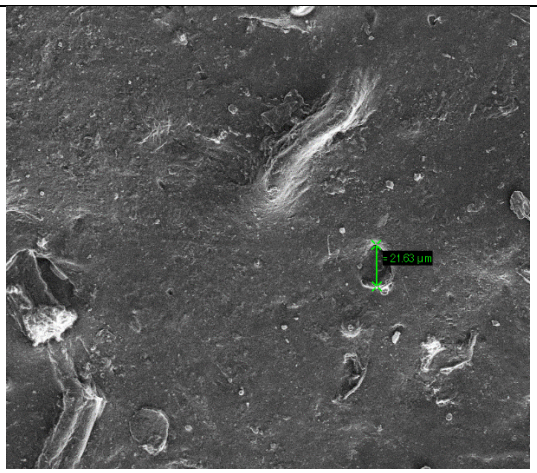

Fig. 15: (PVA with AloeVera with pores)

C. Optical profilometry

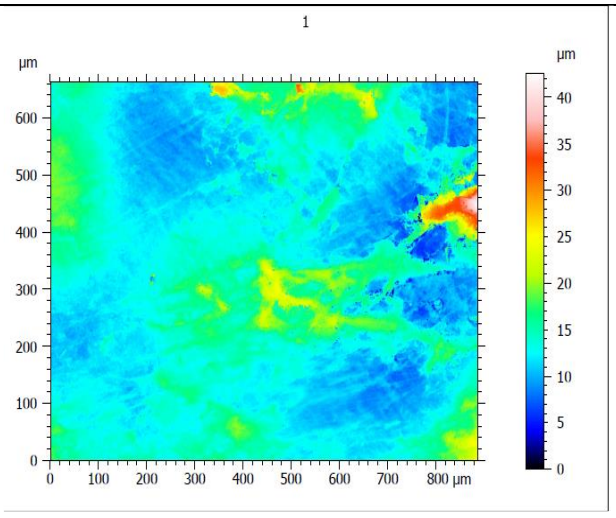

Fig. 16: (S1 Ra value 0.831)

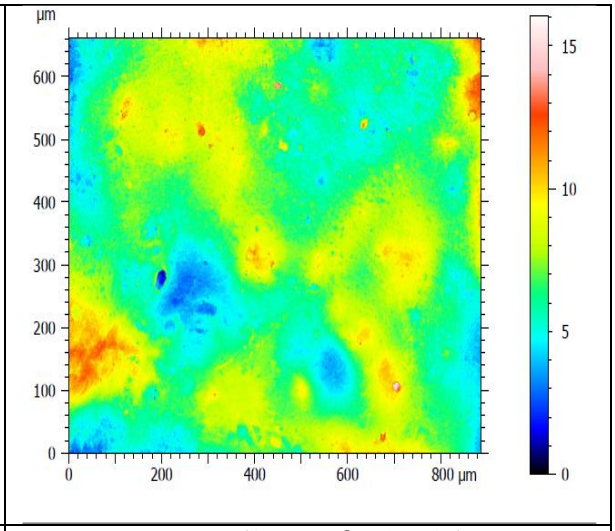

Fig. 17: (S2 Ra value 0.4787)

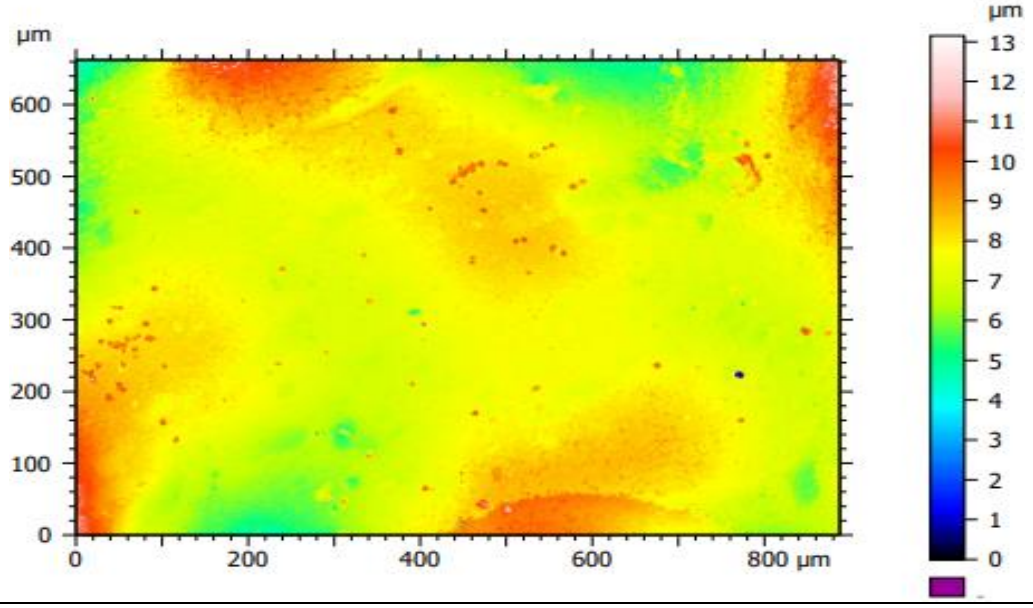

Fig. 18 (S3 Ra µm 0.1907)

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D. Optical profilometry of PVA + Aloe vera films

The developed films S1, S2 and S3 showed roughness values much less than 10 which confirmswith the smoothness of the films to be used for biomedical applications as roughness in films are leads to patient discomfort and irritation at the wound site.

E. TENSILE STRENGTH DETREMINATION

The maximum tensile strength and the percentage elongation were performed on the films using an MTS Bionix machine with a tensile loading rate of 0.2 mm/s. All specimens were cut into a specific dumbbell shape (75 mm long, 4 mm at the middle and 25 mm of measuring segment). A film test was performed in a dry state.

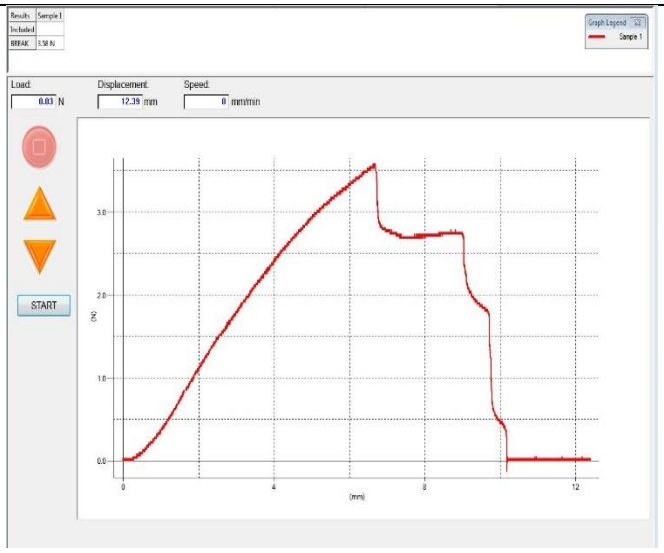

Fig. 19: (S1--TS 3.58N)

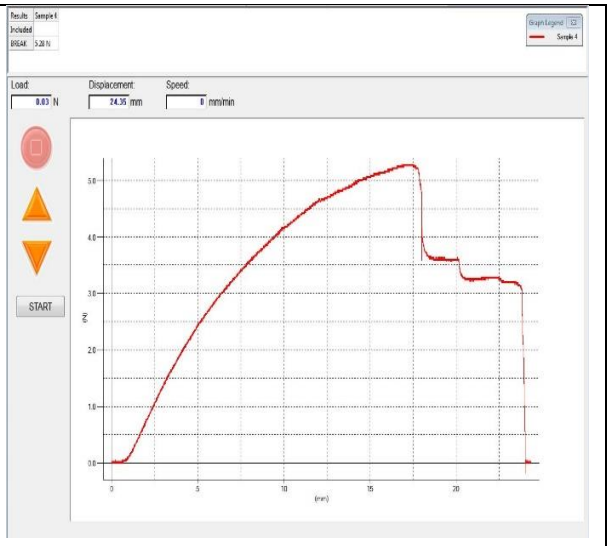

Fig. 20: (S2-TS 5.28N)

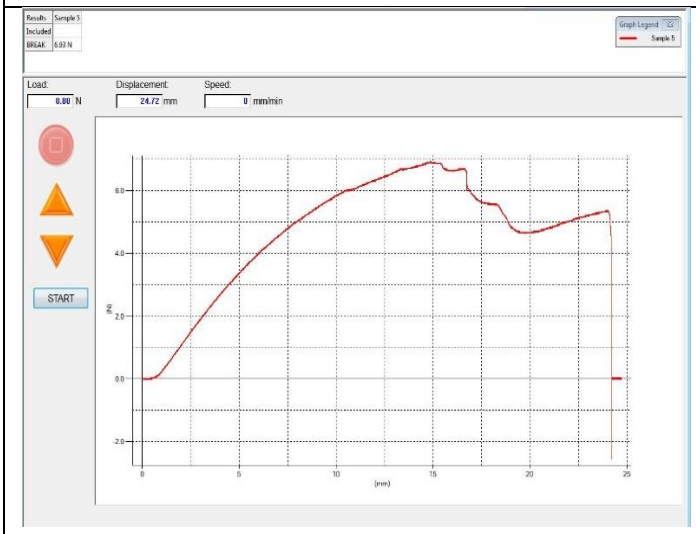

Fig. 21: (S3 -TS 6.93N)

The UTM data obtained from tensile strength study exhibited good mechanical behavior of the developed films. S1 exhibited a tensile strength of 3.58N, S2 exhibited tensile strength of 5.28N and S3 exhibited a tensile strength of 6.93N. These observations confirms that the developed films have sufficient physical strength and will not break under normal handling during packaging, transportation and handling during application.

F. % Elongation study

The percentage elongation study helps to understand the flexibility of the films which also exhibits the internal arrangement of the polymer films developed. The percentage elongation study exhibited elongation of the developed films in the range of 0.47% to 0.95%.

Table 1:

SL.NO	SAMPLE	% ENLONGATION
1	SAMPLE 1	47%
2	SAMPLE 2	93%
3	SAMPLE 3	95%

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G. Cytotoxicity studies

➢ *Cytotocicity studies performed to*

This study used to determine the cytotoxicity effect of test compounds on HDF cell lines by using MTT assay. The samples were Poly vinyl alcohol and Aloe Vera, second sample(S2) were collagen send for Cell lines studies, the cell lines used were Human Derived Fibroblasts(HDF).

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-colored water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley, M. C et al., 1986, Mossman et al., 1983). Excluding test agent ,200 cell suspension was seeded in a 96 well plate at the desired cell density and cells were allowed to develop for 1 day or 24hrs and donated materials were sterilized for 1 hr under UV light before being diluted in 1 ml of DMEM-high glucose media for 1 hour and used as a stock solution. Further test agent was applied and theplate was incubated at 37°C for 24 hours in a 5% CO2 environmentand the plates were removed from the incubator after the incubation period, spent media was removed, and MTT reagent was added to a final concentration of 0.5 mg/mL of total volume .To avoiding exposure to light ,the plate was wrapped in aluminum foil for an incubation period of 3 hrs. 100 l of solubilization solution was added Dimethyl sulfoxide (DMSO) after removing MTT reagent. Dissolution of substances carried out, gentle stirring in a gyratory shaker was performed. Pipetting was used to totally dissolve the MTT formazan crystals on occasion, particularly in region of thick growth.At a wavelength of 570nm, absorbance was measured using a spectrophotometer and an ELISA reader.

In this study, 2 test compounds were evaluated to analyse the cytotoxicity effect on HDF cells. The concentrations of the test compounds used to treat the cells are as follows:

Table 2: Details of drug treatment to respective cell lines used for the study

Sl.No.	Test Compound	Cell line	Concentration treated to cells
1	Untreated	HDF	No treatment
2	Std film	HDF	1 dilution
4	Blank	-	Only Media without cells
5	Sample 1	HDF	1 dilution
6	Sample 2	HDF	1 dilution
7	Sample 3	HDF	1 dilution

VI. OBSERVATIONS & CONCLUSIONS

Table 3: % cell viability values of the test compound, Sample 1 against HDF cell lines. The presented values were the mean of 2 independent individual experiments (n=2)

DDRUG CONCENTRATION	**% CELL VIABILITY**
Untreated	100
Std control (Collagen)	98.8±0.83
S1 (6% AV with 9% PVA)	92.99±2.17
S2	98.31±0.49
S3	99.42±0.12

- The results of cytotoxicity study performed by MTT assay suggests that the given test compounds, Sample1 (6%), Sample2 (12%) and Sample3(18%) were significantly cell proliferative in nature against HDF cells. We can consider both compounds are bio-compatible in nature on HDF cells without causing cytotoxicity in the working dilution respectively.
- The direct microscopic observations of drug treated images of Test compounds after 24 hours of incubation are shown below.

➢ *Collagen and Samples For Cytotoxic Study*

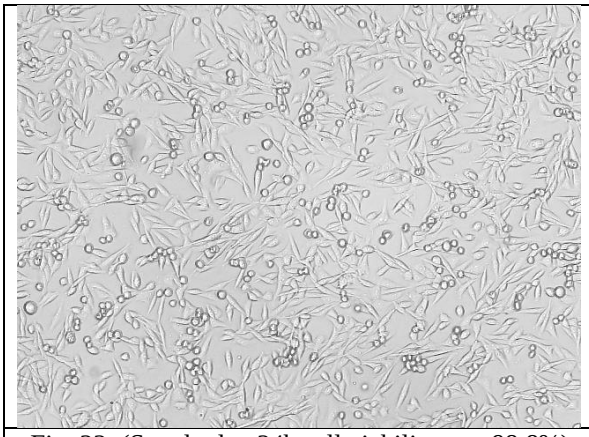

Fig. 22: (Standard at 24h cell viability was 98.8%)

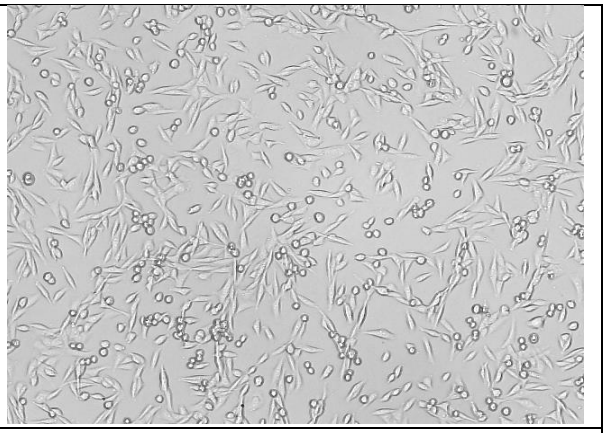

Fig. 23: (Untreated cell viability was 100%)

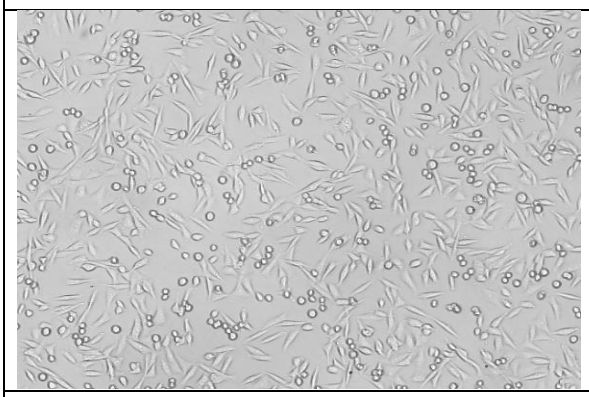

Fig. 24: (S 1 6% cell viability was 92.99% at 24hr)

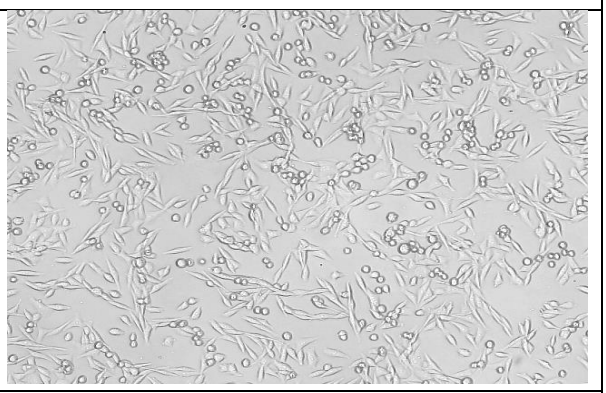

Fig. 25: (S 2 12% cell viability was 98.31% after 24 hrs)

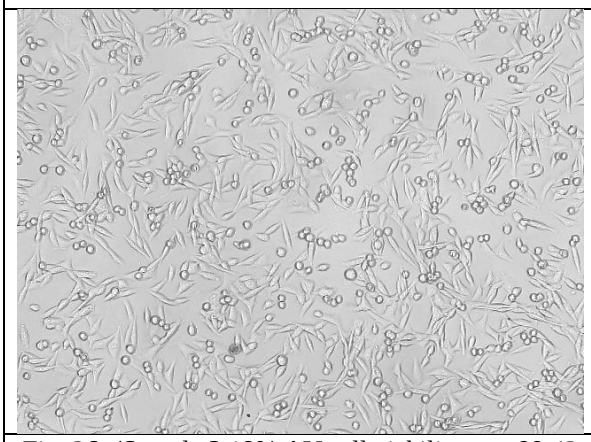

Fig. 26: (Sample 3 18% AV cell viability was 99.42 after 24hr)

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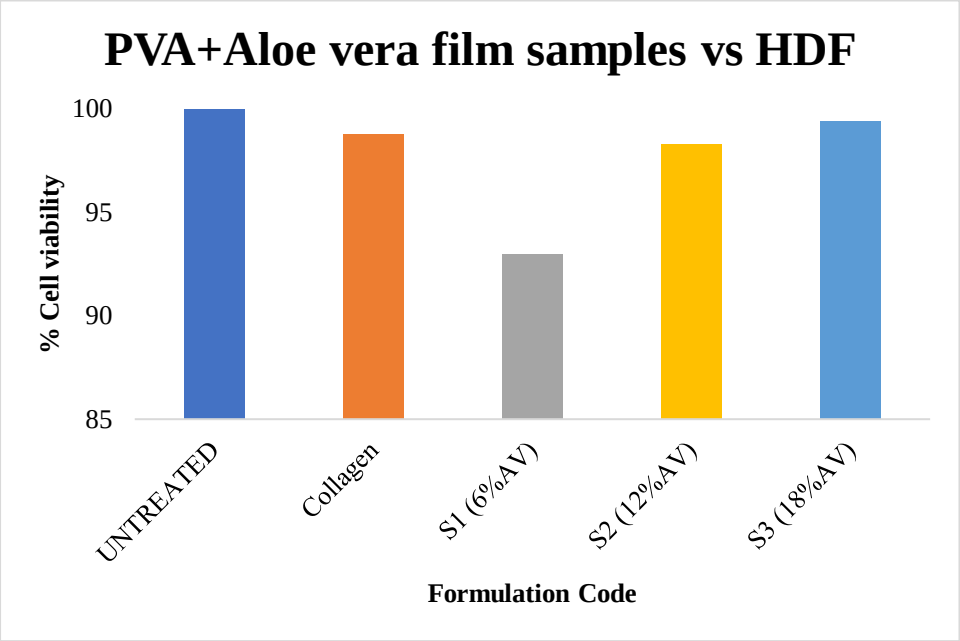

Graph 1:

Table 4: PVA+Aloe vera film samples vs HDF

Exp	UNTREATED	Collagen	S1 (6%AV)	S2 (12%AV)	S3 (18%AV)
Exp-1	100	98.2078853	91.45758662	97.96893668	99.34289128
Exp-2	100	99.39320388	94.53883495	98.66504854	99.51456311
Average	100	98.80054459	92.99821079	98.31699261	99.42872719
SD	0	0.838146805	2.17877159	0.49222542	0.121390314
Std error	0	0.592659289	1.540624166	0.348055933	0.085835914
Average±SD	100	98.8±0.83	92.99±2.17	98.31±0.49	99.42±0.12

VII. CONCLUSION

The MTT assay results suggest that the test compounds, Sample 1, Sample 2 and sample 3 were not cytotoxic in nature on Human dermal fibroblast cells. Aloe vera has medicinal values helps in wound healing. The plant products were always less allergic to human beings. In ancient times Aloe Vera was used in treating burns. Hence Aloe Vera can be used as dressing material. The Polymer which was biocompatible was picked after thorough literature review, the gelatin, PVA was taken into consideration after the trials with gelatin failed. The trials were successful with PVA and Aloe Vera. Mixture of PVA and Aloe Vera had given films and characterization studies were performed. They were 1. Fourier-transform infrared spectroscopy2. Scanning Electron Microscopy3. Tensile strength4. % Elongation 5. Optical Profilometry 6. Cytotoxic Study (MTTASSAY).

FTIR showed no chemical interactions between Aloe Vera and PVA. Scanning Electron Microscopy showed the surface morphology was smooth, homogeneous with pores. Whereas in PVA and Aloe Vera image we can observe roughness which may be due to the addition of aloe vera which has made the topography of the film little rough. Optical profilometry for blend of Aloe Vera and PVA showed surface roughness to be negligible and this the surface is smooth. Tensile Strength exhibited good mechanical behavior of the developed films. % Elongation showed good flexibility for the samples. Cytotoxic Study exhibited excellent cytocompatibility of the developed films with human dermal cell lines and thus it can be concluded that the developed films can be alternative to regularly used wound healing dressings exhibiting reepithelization.

Future prospect of the study:

- Further research work in the physical and chemical characterization studies to be carried out in the preparation of making a novel dressing material.
- Animal trials will be required to prove its biocompatibility and antiallergic property and to observe tissue reaction, re-epithelization and antimicrobial activity.
- Human clinical trials will be required to use the novel dressing material for wound healing that is PVA and Aloe vera as a dressing material after animal trials are successful.

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