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SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF FLAX SEED EXTRACTION LOADED PVA/PECTIN COMPOSITE FILM

AHUJA S*, CHAUHAN DA AND SINOJIA A

Department of Chemistry, Faculty of Applied Sciences, Parul Institute of Applied Science, Parul University Limda-391760 Waghodiya, Vadodara, Gujarat, India

***Corresponding Author: Dr Sonam Ahuja: E Mail: sonam.ahuja82106@paruluniversity.ac.in**

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ABSTARCT

We are creating composite films with two components made of pectin and polyvinyl alcohol and loaded with flaxseed natural plant extract. Pectin and PVA films are created using the solution casting technique. The water absorption of the film increased when pectin and PVA were mixed; thus, glycerine (2ml) was used as the crosslinking agent. The compatibility of these two biopolymers was determined by FT-IR and XRD. The exact ratio of Pectin and PVA was used. In daily life, medicinal plants are frequently utilised to treat a variety of diseases. The extraction of flaxseed has been used as an antibacterial agent. The composite membrane was discovered to have the best antibacterial qualities. Swelling characteristics were also assessed using a gelatin swelling experiment. Based on a minimum inhibitory concentration test, pectin/PVA loaded with flaxseeds also demonstrated strong antibacterial activity against S. aureus and E. coli O157:H7. These findings imply that bio-composites made of pectin and PVA can be employed as unique materials in the biomedical and food packaging sectors. s. We analyse various analysis techniques, including SEM, TGA to examine the properties of the films.

Keyword: Pectin/PVA, Flaxseed, Antibacterial& SEM, TGA, FTIR, XRD

INTRODUCTION:

The creation of innovative wound dressing materials is still of utmost importance today for the treatment of chronic wounds **[1]**. Numerous efforts in the field of cutaneous wound therapy have resulted in the development of a noteworthy method for

producing biomimetic nanofibrous scaffolds using biodegradable and biocompatible polymers **[2]**. Collagen, gelatin, pectin, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and other natural and synthetic polymers have all been utilised extensively in electrospinning for wound healing **[3]**. The utilisation of biodegradable packaging foils is currently insufficient, and researchers are working hard to develop production techniques for high-quality, ecologically friendly systems **[4]**.

1. PECTIN POLYMER: Higher plants include a type of complex polysaccharides called pectin in their cell walls, where they act as a hydrating agent and a cement for the cellulose network **[5]**. Soft plant tissues with rapid development and increased moisture contents have comparatively significant concentrations of pectin **[6]**. It has been debated for a long time whether covalent bonds, in particular, are more significant than calcium-stabilized ionic bonding in maintaining pectin in the cell wall **[7]**. They appear to be involved in regulating the flow of water and plant fluids through the quickly expanding sections **[6]**.

2. PVA POLYMER: Due to its high biocompatibility, polyvinyl alcohol (PVA) hydrogel is one of the most widely used polymer gels in the biomedical field. It is utilised in a variety of biomedical applications, including contact lenses, artificial organs, implants, and wound dressings for the management of wounds **[8]**. The alkyl ammonium ion-exchanged clay is more difficult to distribute in PVA matrix, according to the morphological analyses. They also claimed that clay has a little impact on thermal stability **[9]**.

3. flax seeds (Natural product): One serving of flaxseed provides a good amount of protein, fiber, and omega 3 fatty acids. It may help lower the risk of some cancers, help maintain a health weight, and reduce cholesterol and blood pressure. Depending on whether they are of the golden or brown form, flaxseeds can range in colour from a deep amber to a reddish brown hard shell **[10]**. Flax seeds are a well-known source of a high concentration of polyunsaturated fatty acids. Due to its nutritional makeup, which has favourable effects on disease prevention by delivering health-beneficial components, flaxseed has come to be known as a functional food **[11]**.

4.Antibacterial Drug: Plant "lignans" are powerful healing elements that can be located in the flax seed's fibrous shell. The plant hormone precursors called as lignans have immune-stimulating, antibacterial, antifungal, and antibacterial properties. The metabolism of oestrogen is slowed down by these organic estrogen-like substances **[12]**.

MATERIALS

Pectin is purchased from HI Media Laboratories Pvt. Ltd.-Mumbai (INDIA).

PVA is bought via from HI Media Laboratories Pvt. Ltd.-Mumbai (INDIA).

METHOD

Preparation of Pectin & PVA Film: For the preparation of film, take 40 ml of distilled water in a beaker, placed it on a magnetic stirrer, and stir for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM. 20 minutes later, add 2gm of Pectin and mix until the Pectin has melted. After that 1 gm PVA add for improved Film stability. And then glycerine added as a crosslinking agent to make a mix solution. After that Preparation of Pectin & PVA Film For the preparation of film, take 40 ml of distilled water in a beaker, placed it on a magnetic stirrer, and stir for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM. 20 minutes later, add 1.5g of Pectin and mix until the Pectin has melted. After that 1.5gm PVA add for improved Film stability. and then glycerine was added as a crosslinking agent to make a mix solution. The final mixture was then decanted into a glass petri dish in preparation for film formation and dried in an oven at 40 °C. therefore, the resulting dry film (pectin/PVA) was separated from the petri dish and kept at room temperature 48h overnight. Similarly pure Pectin/PVA film with different ratio is also prepared **[1]**.

Table 1

Ratio	Pectin	PVA
75:25	2gm	1gm
50:50	1.5gm	1.5gm

2: Extraction of flax seeds: To extract proteins, defatted flaxseed is floated in water and extracted at a high pH (pH 9–10 adjusted with base) and certain temperature (often 37–40 °C). After the mixture filter it. After that, taking this mixture as an antibacterial **[10]**.

3: Preparation of drug-loaded antibacterial film: Take 40 ml of distilled water in a beaker, put it on a magnetic stirrer, and stir it for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM to prepare film. Add 1.5 gm of pectin and stir for another 20 minutes or until the pectin has melted. after that add 1.5gm PVA and add a 4-5ml mixture of drug which is made by adding into a 50 ml of pure Distilled water **[10]**.

CHARACTERIZATION

1- FTIR: Equipment: "Perkin Elmer" Spectrum Two or an equivalent triturate 1 to 2 mg of the substance under test with 300 mg of potassium bromide (appropriate for IR analysis), and crush with a 13 mm die at a pressure of around 10 tonnes. With the help of this pellet, you may acquire infrared absorption spectra with wavelengths between 400 and 4000 cm-1 **[16]**.

2- XRD (X-Ray Diffraction): The evaluation of the crystallinity and structure

of solid materials is frequently done using the X-ray diffraction (XRD) technique. In conclusion, the X-ray diffraction phenomena in crystals is the outcome of an unchanging wavelength X-ray scattering process by the electrons of atoms present in the sample **[13]**.

3- SEM (Scanning Electron Microscopy): The scanning electron microscope (SEM) currently employs a wide variety of operating settings to target the required sample volume and advanced modelling approaches to understand the data in order to describe such materials. It also employs cutting-edge imaging techniques to generate new kinds of data **[14]**.

4-TGA (Thermogravimetric analysis): TGA is a material analysis technique that evaluates the mass of such an experiment as a variable of time or temperature under controlled heating. Temperature and weight loss are recorded as the components of the material volatilize over time. TGA testing is a great choice for assessing polymers because it can quantify weight loss at extremely high temperatures. Polymers typically melt before decomposing, at roughly 200°C, however, some can sustain temperatures of 301°C in air and 505°C in inert gases without deterioration. TGA can examine these polymers as well **[15]**.

5- Gelatin Expansion study: Using the variation in diameter of a circular film sample in a 10% gelatin solution, the expansion of the wound dressing film on the wound surface was examined. In general, 10 g of gelatin powder were dissolved in 100 ml of warmed distilled water while being continuously stirred to produce a transparent solution. The gelatin solution was then sprayed over all ratio films with known diameters on a petri dish, and the diameter change was continuously monitored until the sample's diameter stabilised.

4. RESULT AND DISCUSSION

4.1. FTIR

These FTIR tests were performed at PNP Analytical solution, Vadodara (Gujarat, India) with full precision. To find chemical and inorganic contaminants that might contaminate or harm products, Fourier transforms infrared spectroscopy (FT-IR) is utilised. Therefore, FTIR is widely used to identify the first phase of any sample. This allowed for the collection of FTIR spectra for the two different ratio composite films, pectin/PVA (1.5/1.5) and pectin/PVA (2/1), whose band frequency results were as follows: **(Figure 1, 2)**.

4.2. XRD

X-ray diffraction analysis is a technique used in material science to determine the crystallographic structure of a material. This method examines the intensity and dispersion of the rays as they leave the material by exposing the sample to X-rays. **Figure 3** depicts the XRD spectrum of a composite Pectin/PVA film. An X-ray

diffractometer showed a hump at 19.5 (2= 5.0485) in the spectrum, which is consistent with the film's amorphous nature **[13]**.

4.3.SEM:

We used field emission scanning electron microscopy to analyze the film shape (FESEM). The distribution of nanoparticles within the continuous matrix, the homogeneity of the composite, the existence of accumulation, the presence of voids, and the potential orientation of nanoparticles are all information that may be obtained using FESEM. Observations were made on the surface of the Pectin/PVA film following the synthesis **(Figure 4) [14]**.

4.4.TGA:

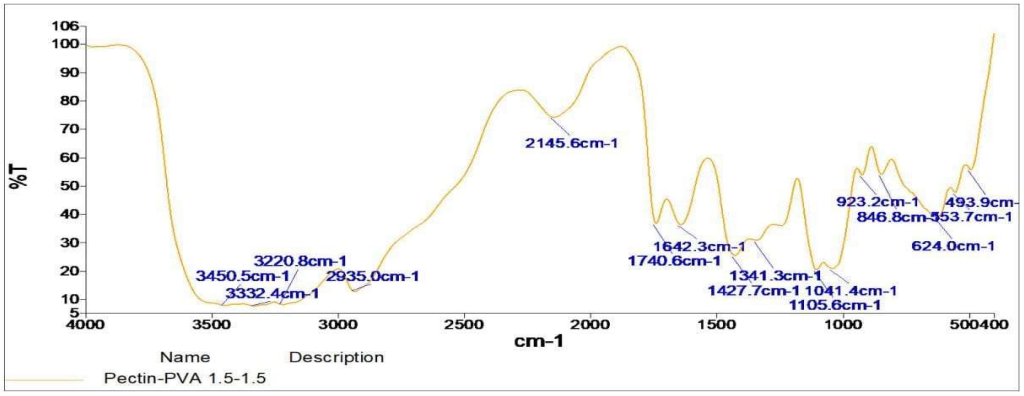
The graph and table of TGA shown above show how stable the gel can be up to what temperature, with the final measurement at 784.97 ◦C, its weight remaining only %. The polymerization of the described gelatin-based compositions was slowed by the addition of the second, more stable component, by 27.699%. The amount of gelatin dispersion in the mixture may be responsible for these outcomes. As a result, the more durable part should serve as a shield to keep the less durable part from deteriorating **(Figure 5) [15]**.

4.5. Antibacterial Activity:

The antibiofilm activity of the flaxseed oil extract was tested. It was chosen specifically for its antibacterial properties. All of the analysed bacterial isolates (*MSSA, MRSA, S. epidermidis,* and *K. pneumoniae*) were resistant to the flaxseed's antibiofilm action. One piece from each of the Pectin/PVA (2.5/0.5) and Pectin/PVA (50:50) films was placed side by side with some distance in between them in a petri dish containing *E. coli* culture. The results indicate the presence of bioactive compounds in pure oregano extract loaded composite films with zones of inhibition measuring 3.2 cm in diameter and 1.4 cm in diameter for pectin/PVA films **(Figure 6)**.

4.6. Gelatin Expansion: Expansion studies are performed to assess the growth in diameter of polymer films. The following expression was used to determine the Expansion ratio (ER):

ER = Dt/Do, where Dt is the diameter at time t and Do is the starting diameter **(Table 2)**.



Sample Details 1

Setting	Filename	Creation Date	Analyst
Value	D:\FTIR_PNP-09\2023\January-2023\12012023\Pectin-PVA 1.5-1.5.5 pnp	12-01-2023 12:32:37 PM	Analyst

Sample Details 1

X-Axis Units	X-Axis start value	X-Axis end value	Number of points	Y-Axis Units
cm-1	4000	400	3601	%T

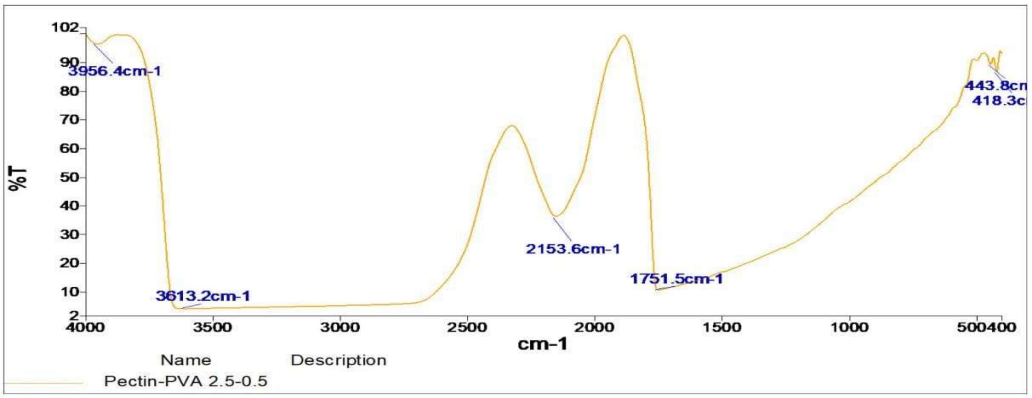
Single Peak Table 1

Peak Number	1	2	3	4	5	6
X (cm-1)	3450.54	3332.35	3220.78	2935.01	2145.59	1740.61
Y (%T)	7.70	7.49	7.99	12.71	74.35	36.83

Single Peak Table 1

7	8	9	10	11	12
1642.27	1427.72	1105.58	1041.38	846.82	623.98
36.38	25.49	20.46	20.84	54.11	38.80

Figure 1: FTIR spectrum of Pectin/PVA composite film with different weight ratios: (a) Pectin/PVA =1.5/1.5gm



Sample Details 1

Setting	Filename	Creation Date	Analyst
Value	D:\FTIR_PNP-09\2023\January-2023\12012023\Pectin-PVA 2.5-0.5.5 pnp	12-01-2023 12:22:56 PM	Analyst

Sample Details 1

X-Axis Units	X-Axis start value	X-Axis end value	Number of points	Y-Axis Units
cm-1	4000	400	3601	%T

Single Peak Table 1

Peak Number	1	2	3	4
X (cm-1)	3613.19	2153.58	1751.45	418.30
Y (%T)	3.99	36.43	10.46	86.94

Figure 2: FTIR spectrum of Pectin/PVA composite film with different weight ratios: (b) Pectin/PVA =2.5/0.5gm

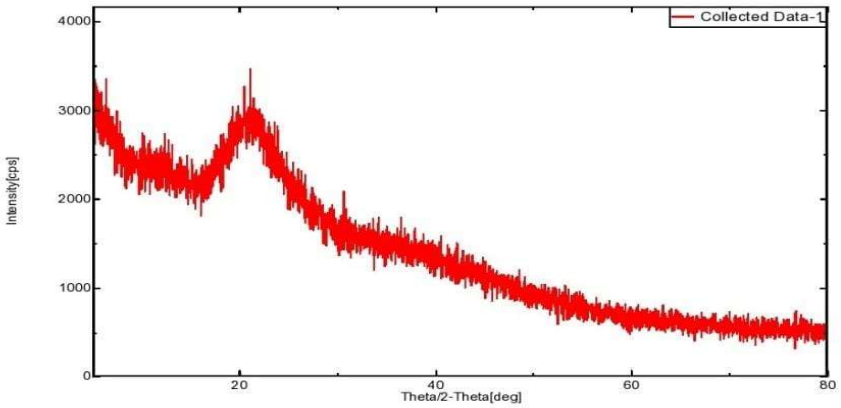


Figure 3: X-ray graph of composite film

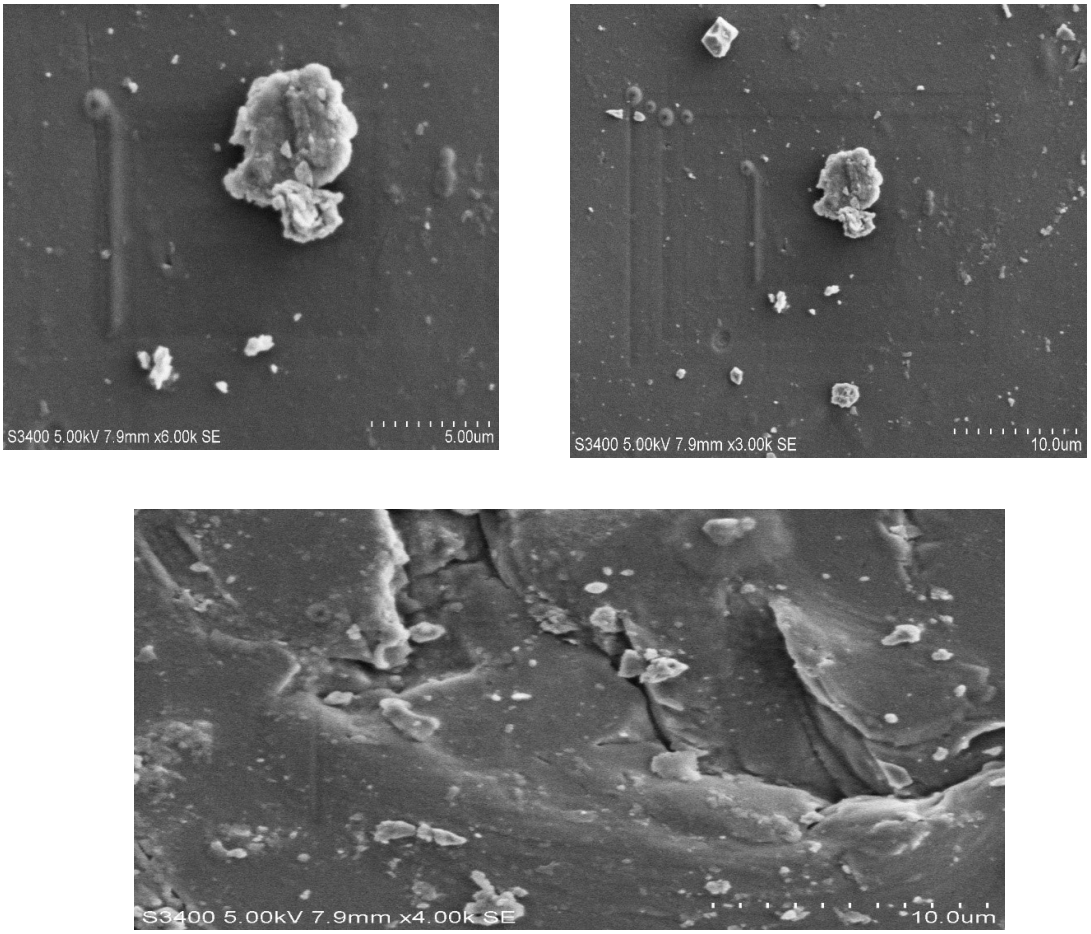


Figure 4: SEM images of the surface of Pectin/PVA film

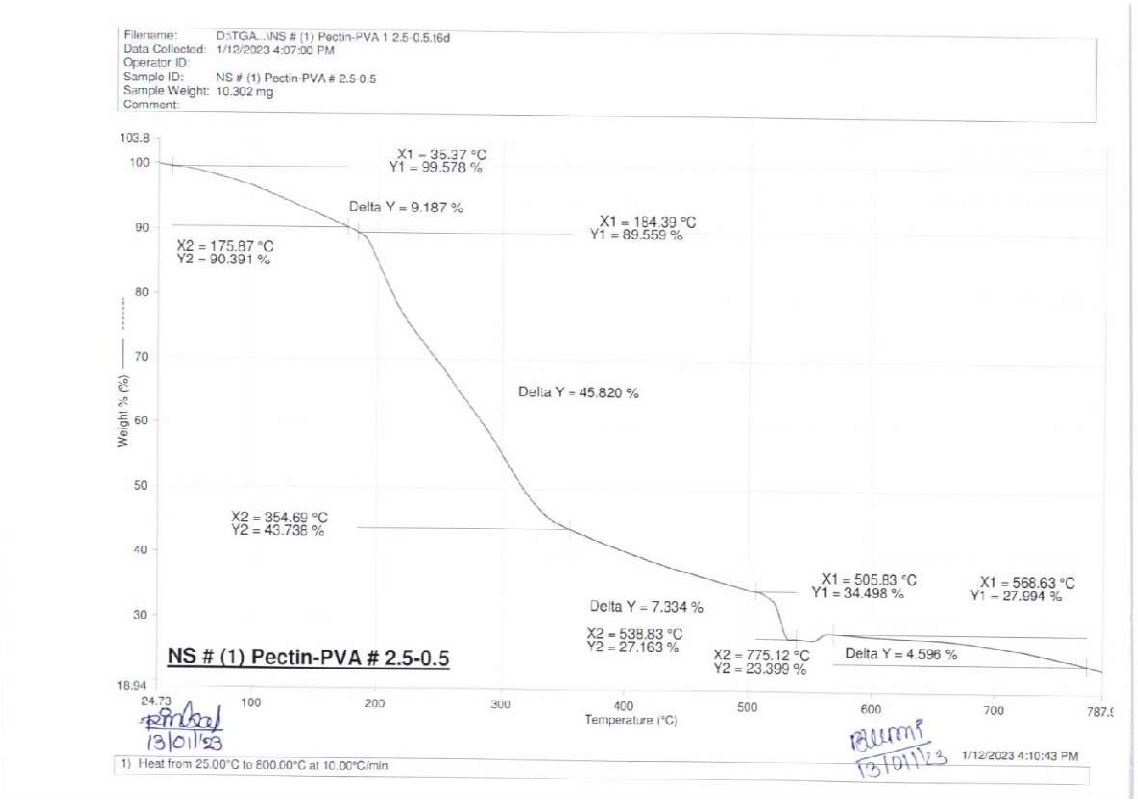


Figure 5:TGA of Pectin/PVA composite film

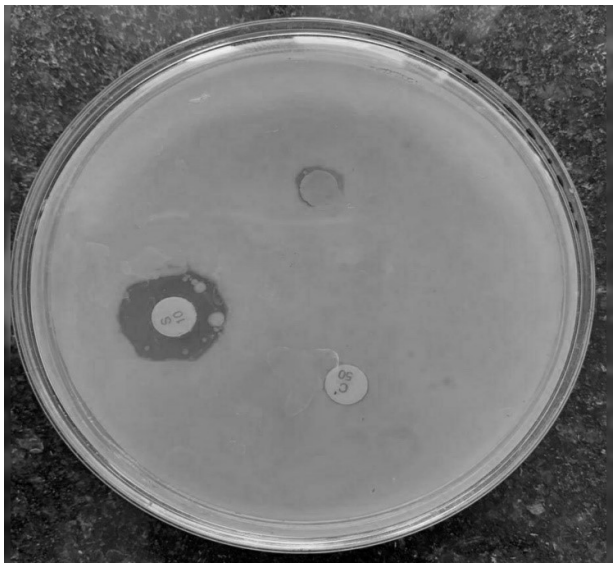


Figure 6: Zone of Inhibition of flax seed extraction loaded pectin/PVA Composite film

Table 2: Gelatin Expansion study table

No.	Ratio	0min	15min	30min	45min	60min	24hr
1	2g pec/1gm PVA	2cm	2.1cm	2.1cm	2.2cm	2.3cm	2.4cm
2	1.5g pec/1.5gm PVA	2cm	2.2cm	2.2cm	2.3cm	2.4cm	2.5cm
3	2.5g pec/ 0.5gmPVA	2cm	2.2cm	2.3cm	2.4cm	2.5cm	2.5cm

CONCLUSION:

The solvent casting technique has been used to produce PVA/pectin blended films with various component ratios. In-depth research has been done on the influence of PVA molecular weight and pectin type on their physicochemical and mechanical properties. Blended PVA/pectin films were also crosslinked with glycerine in order to strengthen the film structure and improve their stability in cultural media. The best glycerine concentration for obtaining samples that are conducive to Cell attachment has been identified. The obtained PVA/pectin films with attached Cells therefore appear to be potential materials for use in regenerative medicine as a component of innovative cell-containing tissue engineered wound dressings.

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