Preparation and Characterization of Polyvinyl Alcohol-Alginate(PVA-SA) Nanoscaffold for Tissue Engineering Application

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Abstract :- In recent years , due to the emergence of nanotechnology , researchers are highly focused on unique properties of nanoscale materials for advancing the medical field. Surgical treatment of burn injuries suffers from a limited availability of engraft able skin and is particularly suited to tissue engineering applications. Nanomaterials namely composites, metals, ceramics, polymers nanostructure surfaces features are used to treat skin burns. So we use both synthetic and biopolymers to preparing the scaffold .Nanoscaffolds, has been recognised as an efficient technique for tissue regeneration and highly recommended for skin burns. Polymers are commonly used for skin burn treatment due to their mechanical and chemical properties. Here we propose a simple method of developernent of PVA-SA nanoscaffold with good thermal ,chemical properties .since sodium alginate(SA) is a natural polymer ,it remains biocompatible and toxicity. The SEM images represents the pore's nature thereby recommend for the drug to carried. Based on the viscosity of the solution ,PVA maintains the chemical and physical resistance of the scaffold. The above result shows that PVA-SA scaffold is highly recommend for a drug delivery support tissue for skin burn application.

***Keywords:** Polyvinyalcohol(PVA), Sodiumalginate(SA), Nanoscaffold, Blood compatability , Skin burns.*

1. INTRODUCTION

In recent years , due to the emergence of nanotechnology,reseachers are highly focused on unique properties of nanoscale materials for advancing the medical field. Surgical treatment of burn injuries suffers from a limited availability of engraft able skin and is particularly suited to tissue engineering applications. Nanomaterials namely composites, metals, ceramics, polymers nanostructure surfaces features are used to treat skin burns. So we use both synthetic and biopolymers to preparing the scaffold .Nanoscaffolds, has been recognised as an efficient technique for tissue regeneration and highly recommended for skin burns.skin is the largest organ of the body, it covers 15% of the body weight. Skin can be damaged by variety of factors including skin burns, skin cancer, wrinkles etc.. Here wound healing for skin burns are discussed. Skin burns can occur by fire , heat, electricity, condensation, sun exposure etc... There are three categories of skin burns and they are First degree, second degree and third degree.Among the type of these skin burns Third degree burn affects the Hypodermis layer of the skin and it makes severe damage to the skin. The second degree burn may turn into third degree burn by the occurrence of infection.As a result of coagulation necrosis subcutaneous fat and dermis layer get destroyed. Blood clot occurs in blood vessels beneath the skin. Healing can take much longer because of the burn affects subcutaneous fat. Third degree burns occurred by charted skin.

To conquer this issue ,present day dressings as vehicles for conveying remedial operators in wound sites have been connected in the form of films,scaffold, sponges ,foams ,hydrogels and skin graft. With a specific end goal to grow such a structure, careful selection of the three primary part is required:1) Scaffold , 2)growth factors and 3)cells. They are clarified as 3D permeable strong biomaterials that can control cell behaviour such as migration , proliferation, differentiation , and extracellular matrix(ECM) deposition.Scaffold design and manufacturing are important areas of biomaterial research , and they are also well being subjects for tissue engineering and regenerative medicine research. Scaffold plays a particular role in tissue regeneration and repair .Scaffold materials can be synthetic or biologic , degradable or non degradable rely on the particular purpose .The properties of polymers determined by composition , structure and process of their constituent macromolecules it can be grouped into various characteristics in terms of their structural , chemical and biological features, For example, Ceramics, Glasses ,Polymers etc...There are two types of polymers used as a biomaterial and they are synthetic polymer and natural polymer.Poloymers used for fabricate scaffolds are chitosin, PVA, PLA, PLGA, PU, hydrogels, SA, PLLA, PGA etc .Methods for preaparing scaffolds are solvent casting,salt leaching, lypophilisation, electrospinning etc.In this study, make use of SA and PVA were selected as wound dressing because of their good characteristics and preparing scaffold in both solvent castinng and salt leaching and lypophilisation.The combination of organic and inorganic polymers represents the request in terms of capable of mechanical and biological performance in

tissue. The properties of the SEM, UV, cytotoxicity, viscosity and hemocompatability were investigated successfully.

2.MATERIALS AND METHODS

2.1 Materials and Reagents

Poly vinyl alcohol(PVA-degree of polymerisation(1700-1800)) and sodium Alginate(SA) was purchased from spectrum(spectrum reagents and chemicals pvt.Ltd, Edayar, Cochin). DMEM medium, Fetal Bovine Serum(FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethylsulfoxide) and MTT(3-4,5dimethylthiazol-2yl-2,5- diphenyl tetrazolium bromide) (5mg/ml) were from Sigma,(USA),1XPBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson(India).

2.2 Preparation of PVA/SA scaffold

2.2.1 Lypophilisation method

3 g (for a 10**%** wt/vol solution) or 6 g (for a 20% solution) of PVA powder was dissolved into 50 ml of deionised water by using magnetic stirrer.Then the solution is heated at 120˚ for 1 hour and allowed to cool at room temperature.Sodium Alginate (50 wt% of PVA) were mixed with the 25 ml of deionised water by using stirrer.Then the SA solution were added with PVA solution and mixed for 5 minutes at 500 rpm.The solution were allowed to settle for 1 minute, the overhead mixer was set to 150 rpm and 6 ml of dichloromethane (DCM) was added drop-wise as the mixture was stirred for 3 minutes.The mixing speed increased to 300 rpm due to the higher viscosity of the polymer solution.Finally, the solution were poured into the glass Petri plate and subjected to freeze/thaw cycles which consisting of 23 hours at -25˚ C followed by one hour at25˚ C follows for 3 cycles.

2.2.2 Solvent casting and salt leaching

The above procedure will be followed until the mixture pour into the petri plate and then porogens (Nacl) dispersed over the mixture.This will kept undisturbed for 48 h and scaffold is finally formed.

3. CHARACTERISATION

3.1 Viscosity

Viscosity was measured by using DV-E Viscometer (brook field) in polymer lab, St.joesph College,Trichy.It is used to determine the viscosity ranges of polymers.

3.2 Scanning electron microscope

The surface and internal structure of the membrane samples were investigated by Analytical SEM (type: JEOL JSM 6490 LS,Trichy) with 10kV voltage.It is used to analyse the surface roughness,porosity and pore size. This membranes were dehydrated by freezr-Dryer.

3.3 FT-UV

Fourier transform ultra violet (FT-UR) is used to find out the biological and chemical componds of materials.

3.4 Cytotoxity evaluation

3.4.1 Cell culture

Vero cells (African green monkey kidney cells) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS) , 100 µ/ml penicillin and 100 µg/ml streptomycin ,and maintained under an atmosphere of 5% CO_2 at 37˚C.

3.4.2 MTTAssay

The Scaffold sample was tested for invitro cytotoxicity, using Vero cells by(3- 4, 5-dimethylthiazol 2-yl 2, 5-diphenyl tetra zolium bromide) (MTT)assay. Briefly, the cultured Vero cells were harvested by trypsinization, pooled in a 15 ml tube.Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200µl) into 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 hours at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the Scaffold sample in a serum free DMEM medium. The scaffold sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24h

After the incubation period, MTT (20µl of 5mg/ml) was added into the well and the cells incubated for another 24 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220µl) were aspirated off the wells and washed with 1XPBS (200µl). Furthermore, to dissolve formazan crystals , DMSO (100µL) was added and the plate was shaken for 5min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific,USA) and the percent Age cell viability and IC 50 value was calculated using Graph Pad Prism 6. 0 software (USA).

4 RESULTS

4.1 Scaffold

The final scaffolds prepared by solvent casting and salt leaching (a) and (b) lyophilisation methods and their pictures are given below

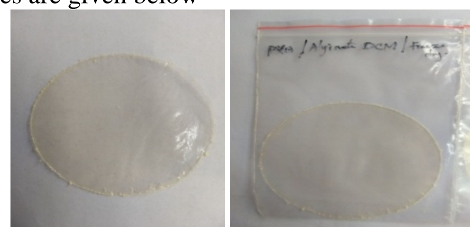


fig (a) fig (b)

4.2 Viscosity

By blending 15% PVA and 2% SA, smooth mats were obtain by solvent casting and salt leaching method with balanced equilibrium. An increase in viscosity of the solutions was observed as the concentration of SA gets increased. Thickness of the fibrous mat is measured about 0.15 mm.

4.3 Scanning electron microscope

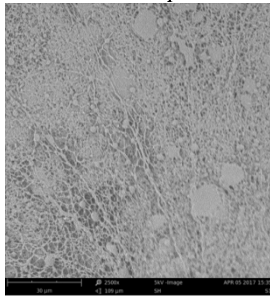


fig .(a)

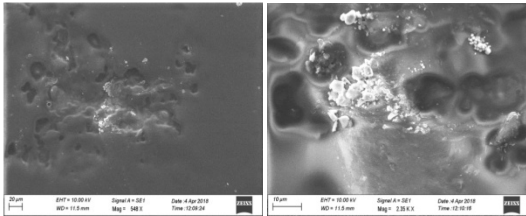


fig.(b) fig.(c)

The surface morphologies of the samples were visualized under JEOL JSM 6490 LS scanning electron microscope.The image indicates the surface of SA/PVA nanopores.These images were used to examine approximate surface properties and clusters formation. This SEM images reveals the smoothness of scaffold surface.pore size remains lager within the sample is shown in fig (c). porosity is given in image (a).this shows that the sample has high porosity than their pore size which is an essential for scaffold preparation.clusters in the sample region is due to the viscosity changes with in the solution.the smooth surface indicates that the scaffold can be recommended as a drug carrier for tissue engineering applications.

4.4 FT-UV

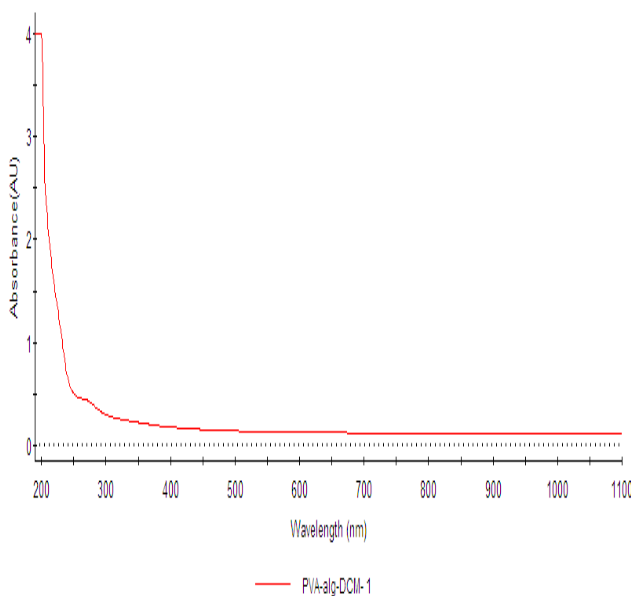


fig (a)

VISCOSITY			
Sodium Alginate (SA)		**2% SA : 15% PVA**	
Concentration (%)	Viscosity (cPs)	Blending ratio	Viscosity (cPs)
1	0	01:01	247.5
2	16.67	01:02	1140.17
4	294.167	02:01	144.17
Poly Vinyl Alcohol (PVA)			
Concentration (%)	Viscosity (cPs)		
10	630.83		
12	1481.67		
15	9936.67		

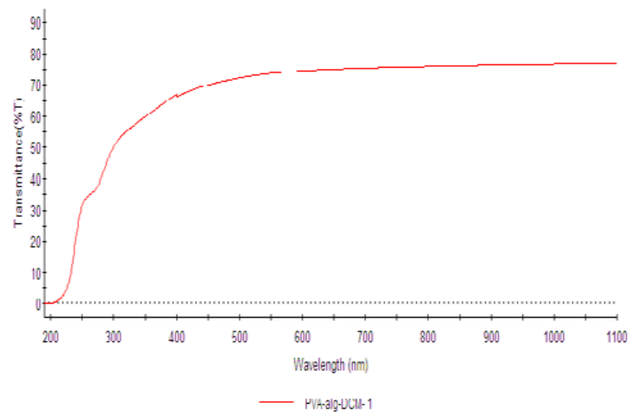


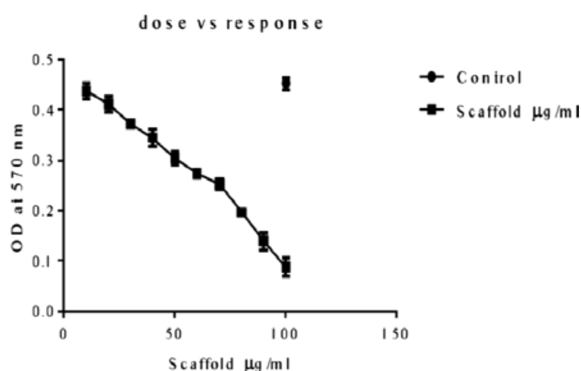
fig (b)

Fig (a) & (b) represents UV absorbance and transmittance of our sample. The results interprets that around 300,340 wave length indicates the presence of C=N and N=N bonds. The presence of Carbon and nitrogen are due to PVA and sodium alginate compounds.Appreance of a light peaks nearby 400 wavelength indicates pyridine, acetone composition due the DCM solvent present in our sample. This indicates the organic compounds within the scaffolds are carbon, nitrogen, acetone pyridine and other polymeric substances within the scaffold. The level of carbon and nitrogen is minimum that shows the scaffold is less toxic which is proved in cytotoxic test.

4.5 Cytotoxity

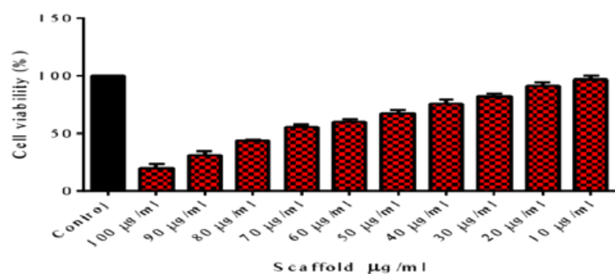
4.5.1 A.OD value at 570 nm control meanOD value:0.452

S. No	Tested Scaffold sample concentration(µg/ml)	OD Value at 570 nm (intriplicates)		
1	Control	0.439	0.462	0.456
2	100µg/ml	0.093	0.067	0.105
3	90µg/ml	0.142	0.156	0.122
4	80µg/ml	0.201	0.189	0.200
5	70µg/ml	0.241	0.263	0.252
6	60µg/ml	0.282	0.267	0.273
7	50µg/ml	0.319	0.299	0.297
8	40µg/ml	0.328	0.345	0.361
9	30µg/ml	0.364	0.372	0.382
10	.20µg/ml	0.412	0.396	0.428
11	10µg/ml	0.439	0.451	0.420



4.5.2 B.Cell viability(%)

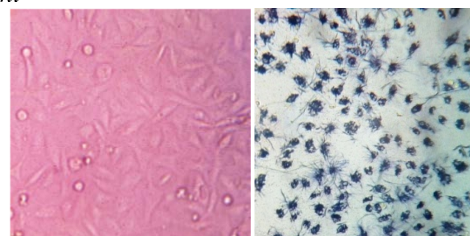
S.No	Tested Scaffold concentration (µg/ml)	Cell viability (%) (intriplicates)			Mean Value (%)
	Control	100	100	100	100
	100µg/ml	20.57	14.82	23.23	19.54
	90µg/ml	31.41	34.51	26.99	30.97
	80µg/ml	44.46	41.81	44.24	43.50
	70µg/ml	53.31	58.18	55.75	55.74
	60µg/ml	62.38	59.07	60.39	60.61
	50µg/ml	70.57	66.15	65.70	67.37
	40µg/ml	72.56	76.32	79.86	76.24
	30µg/ml	80.53	82.30	84.51	82.44
	20µg/ml	91.15	87.61	94.69	91.15
	10µg/ml	97.12	99.77	92.92	96.60



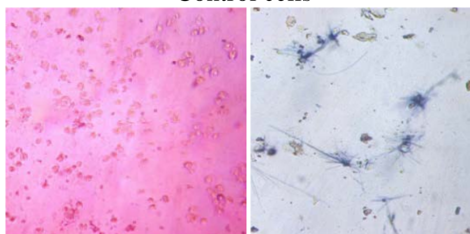
4.5.3 C.IC 50 Value of tested Scaffold Sample:58.61µg/ml

Log (inhibitor) vs .normalized response--Variable slope	
Best-fit values	
Log IC 50	1.768
Hill Slope	-3.137
IC 50	58.61
Std.Error	
Log IC 50	0.01213
Hill slope	0.2906
95% Confidence intervals	
Log IC 50	1.743 to 1.793
Hill slope	-3.732 to -2.541
IC 50	55.35 to 62.06
Goodness of fit	
Degrees of freedom	28
R square	0.9378
Absolute sum of squares	1857
Sy.x	8.144
Number of points	
Analyzed	3 30

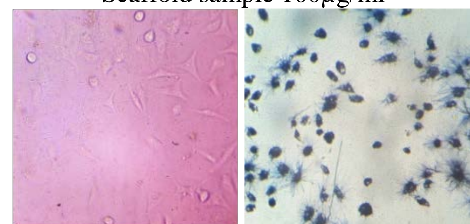
4.5.4 D.Formation of formazan crystals in control cell sand Scaffold treated cells Before MTT treatment After MTT treatment



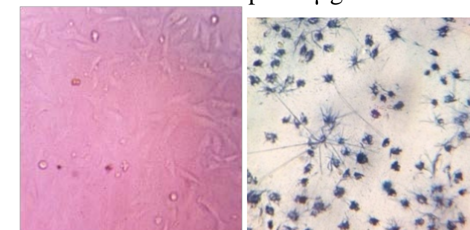
Control cells



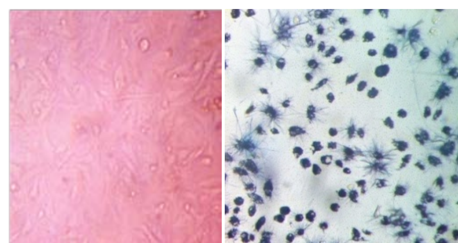
Scaffold sample 100µg/ml



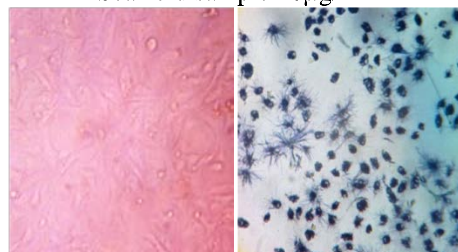
Scaffold sample 80µg/ml



Scaffold sample 60µg/ml



Scaffold sample 40µg/ml



Scaffold sample 10µg/ml

5. CONCLUSION

Prepared scaffolds were proved as safe by the result of cytotoxity are concluded.In future, it has been tested for hemocompatability and biodegradation rate.

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