Original Article

Polyacrylamide Grafted Gum Acacia (GA-g-PAM) Graft Copolymer as Efficient Polymeric Scaffold

Trishna Bal[1,*], Priyashi Singh[1], Aditya Dev Rajora[1], Sandeep Garg[1], Barnali Dasgupta Ghosh[2], Ashok Kumar Pattnaik[1]

[1]Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, INDIA.
[2]Department of Chemistry, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, INDIA.

ABSTRACT

Background: Natural polysaccharides are mostly not stable in their original form but their biodegradable properties can be beneficial for use as polymeric scaffolds for tissue engineering. Microwave irradiation assisted grafting synthesis being an easy and quick method enhances stability of these natural polysaccharides. **Materials and Methods:** Initially the optimization of the redox initiator ammonium per sulfate (APS) along with the monomer concentration acrylamide (AM) was done by % grafting efficiency (%GE) for getting the optimized grade of graft copolymer (GA-g-PAM). The microwave time was kept constant with the concentration of the polymer Gum acacia (GA). Different analytical techniques were used for the characterization of optimized grade G6 like Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), X-ray diffraction(XRD) and Nuclear magnetic resonance (NMR) which confirmed successful grafting reactions and scanning electron microscopy (SEM) was utilized to analyze the interior architecture as well the cell proliferation on to the polymer surface. The optimized grade G6 was inserted under the skin hypodermis for tissue proliferation. **Results:** The % grafting efficiency (%GE) of optimized grade of Polyacrylamide grafted Gum acacia (GA) graft copolymer (GA-g-PAM) was 94.54%. Histolology studies of local tissue of the test mice revealed that the polymer material after insertion onto the mice skin enhanced the cell proliferation as there were evidences of more collagen as well as fibroblast growth in test animal local tissue than in comparison to control mice. **Conclusion:** Thus, results indicate that polyacrylamide grafted gum acacia graft copolymer (GA-g-PAM) is sufficiently biocompatible and suitable as versatile material for tissue engineering purpose.

Key words: Graft copolymer, Microwave assisted synthesis, Tissue engineered scaffold, Gum acacia, Acrylamide.

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Correspondence:
Prof. Trishna Bal
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, INDIA.
Phone: +91- 7004273537
Email id: trishna.bal@gmail.com

INTRODUCTION

A scaffold is a support system which enables the proliferation of cells by mimicking the extracellular matrix of the cell and even develops the cells into tissues and organs. Many studies revealed that without a proper template for tissue growth, the cells develop into a 2D structure which has less mechanical strength and thus show poor stability.[1] Biocompatible natural polymers as polymeric scaffolds have been used traditionally for the cell proliferation and tissue growth. Thus, many naturally derived polymers are widely used for cellular regeneration and leading to the development of tissues.[2] Natural plant exudates either in the form of mucilage or gums are widely used as scaffold for the preparation of nanoparticles for various applications.[3] Polymeric scaffolds are widely used in various applications like regeneration of bone cells, muscle regeneration etc.[4] Graft copolymerization process involves the modification of natural polysaccharides by use of synthetic polymer or monomer sustaining the physico-chemical property of both natural and synthetic polymer.[5,6]

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Besides this, such modifications in the natural polymers increase their shelf life and prevents from instant biodegradation. The use of graft copolymers as tissue engineered polymeric scaffold, form 3D structures and this make them interact and mimic the living cells, providing cytocompatibility.[7] Moreover, the natural polysaccharides are more preferred because of their abundance in nature and their inert properties. The acrylamide (AM) monomers have varied applications in the field of drug delivery, flocculation, stabilizing agents etc.[8] In the recent past, there are some literature on the use of graft copolymer of gum acacia (GA) with AM as drug delivery system,[9] but till now, no literature is present on the use of gum acacia graft copolymer with AM as tissue engineered polymeric scaffold. Thus the present research is based on the preparation Polyacrylamide grafted Gum acacia (GA) graft copolymer (GA-g-PAM) and its utilization as polymeric scaffold for tissue engineering purposes.

MATERIALS AND METHODS

Gum Acacia (GA) (CDH, India), Acylamide (AM) and Ammonium Per sulfate (APS) (SD Fine chemicals India), Acetone (Rankem, India) were used as received.

Synthesis of Gum acacia grafted acrylamide (GA-g-PAM) graft copolymer

Gum acacia grafted acrylamide graft copolymer (GA-g-PAM) was prepared by microwave assisted synthesis utilizing ammonium persulphate (APS) as an initiator in aqueous medium. Different reaction parameters which include APS concentration, monomer AM and polymer GA concentration were varied to get best grade as shown in Table 1. Initially, the polymer GA was dissolved in water in a 100ml glass beaker and AM was added to this solution and stirred and maintained at 50°C in a magnetic stirrer for 15 min till the polymer is completely diffused and solubilized in water. Later, APS was added to the above solution and stirred for another 15 min maintained at the same temperature and microwaved for 40 sec till gel consistency. The reaction vessel was immediately taken out and placed in an ice bath and then precipitated in presence of acetone to remove any unreacted monomer, GA, APS and any other byproducts if so formed.[8] Reaction scheme is detailed out in Scheme 1. The final precipitated product is dried in a hot air oven till to get a constant weight and powdered to fine powder. The different grades were optimized based on % Grafting efficiency (%GE)[8] using the formulas given in Equation 1.

$$\%GE = \frac{\text{Weight of Graft Copolymer synthesized} - \text{Weight of Polymer taken}}{\text{Weight of Monomer}} X100 \quad [1]$$

Characterization and analysis

Graft copolymer G6 and pure polymer GA and monomer AM were characterized using Fourier Transformation Infrared Spectroscopy (FTIR) (Shimadzu, FTIR-8400S), Differential Scanning calorimetry(DSC-60 Shimadzu),[10] X-ray diffraction (XRD).[10] and NMR (Nuclear magnetic resonance)

FTIR spectroscopy

FTIR of pure GA, AM and optimized grade of GA-g-PAM graft copolymer (G6) was done for the confirmation of the functional groups present in the samples. About 10mg of the sample with KBr was analyzed and % Transmittance was recorded between 4000 cm^{-1} and 400 cm^{-1} using FTIR -8400S (Shimadzu, Japan).[11]

XRD (X-Ray Diffraction)

The X-ray diffraction spectroscopy was done for Acacia Gum and G6. The sample were diffracted for 2θ at 2°/min value ranging from 20°-80° and chart speed of 2°/2cm/2q using Bruker AXC D8 Advance, Germany.[12]

Thermal studies

The thermal stability of GA, AM and G6 were analyzed utilizing differential scanning calorimetry (DSC-60 Shimadzu). 30 microlitre aluminium pans are utilized with sample mass at 2°C min^{-1} heated at 25°-80°C. For reference an empty pan is utilized.[13]

Surface morphology

The surface morphology and inner architecture of the optimized graft copolymer G6 and pure polymer GA was observed by using SEM (Scanning electron microscopy), Model Jeol JSM-6390LV, where the samples were placed in a copper stub and coating was done using gold sputtering technique.[14]

^{13}C NMR

^{13}C NMR was done for G6 and physical mixture of GA with AM, at 75 MHz to the ceramic rotor of JEOL CEX-400 spectrometer (Peabody, MA, USA).[8]

Insertion of Polymeric scaffold

Male mice housed in standard conditions at controlled temperature of 25°C and light/dark cycles of 12/12 hr duration were used for the insertion of polymeric sample. The animals were divided into two groups, i.e. standard (cotton pellet) and test (cotton ball coated with graft copolymer solution) consisting of six number each in both the groups. The anaesthetized mice skin was shaved and sanitized with povidone-iodine solution

and a clean incision was made on it, taking care of not rupturing any microcirculation below the skin and the sterilized polymer scaffold was inserted carefully and stitched off the skin with biodegradable suture thread. After a span of 7 days, the scaffold was carefully removed and also the local application tissue was isolated for the histological studies.[15] After collection, the carcasses were disposed by burial.[16] The entire animal experiment has been approved by Institutional animal ethical committee of Birla Institute of Technology, Mesra with an approval number 1972/PH/BIT/102/20/IAEC.

Histopathological procedure

Local site's tissue was removed and dipped in a formalin solution of 10% for 5 days and then mounted in paraffin wax and sectioned at 3µm thickness and later deparaffinized and mounted in the glass slide, stained by Hematoxylin – Eosin. Optical microscope (Leica, DME) was used for capturing the pictures.[15]

RESULTS AND DISCUSSION

Synthesis of GA-g-PAM

Microwave irradiation assisted free radical reaction mechanism was used for preparation of various grades of graft copolymer using redox initiator and the optimization was based on the % GE with the varying amount of APS and AM. Initially the APS content was varied keeping all the other parameters constant to get the highest %GE. Later the concentration of AM was varied to the get the highest % GE and that grade which showed highest %GE in both these parameters was considered as the best grade and that was found to be grade G6 as shown in Table 1.

Characterizations

FTIR spectroscopy

The FTIR Spectra along with the wave number and corresponding functional groups of GA, AM and G6 (GA-g-AM) is shown in Figure 1 and Table 2. In case of GA a sharp absorption peak at 3490 cm^{-1} indicates the hydrogen bonded O-H stretching band. Additional characteristic absorption bands of GA appear at 2931 cm^{-1} and 1424 cm^{-1} due to C-H stretching and O-H bend respectively. One peak at 1618 cm^{-1} is attributed to COO^- group. In AM two peaks one at 3348 cm^{-1} and the other at 3201 cm^{-1} are attributed to N-H stretching. Secondary amides appear at 1686 cm^{-1}. Further there is one more additional band at 1419 cm^{-1} where COO^- group symmetrical vibrations indicate polyacrylamide hydrolysis. The presence of all the characteristic peaks of GA and AM shows that the grafting has occurred successfully on the polymer backbone of GA on AM. In G6 (GA-g-AM), characteristic peak of gum acacia, O-H stretching is present at 3490 cm^{-1}. Also the characteristic peak of AM is present at 1419 cm^{-1} . This confirms the grafting reaction.[8,17]

XRD (X-Ray Diffraction)

The X-ray diffraction pattern of GA as shown in Figure 2, reveals that the material have a broad diffraction pattern, thereby indicating that the pure polymer is amorphous in nature, whereas in case of AM, the diffraction pattern indicates the material to be completely crystalline in

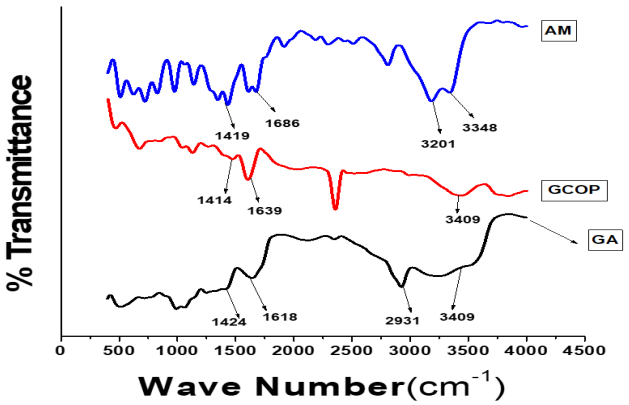


Figure 1: FTIR Spectra of GA (Gum Acacia) AM (Acrylamide) G6 (GA-g-AM).

Table 1: Composition of Gum acacia grafted acrylamide (GA-g-PAM) graft copolymer.

Grade	Wt. of Polymer	Wt. of Monomer	Wt. of (APS)	% Yield	Microwave Time	% GE
G1	500mg	5g	200mg	3.896	40sec	67.92
G2	500mg	5g	250mg	3.352	40sec	57.04
G3	500mg	5g	300mg	4.721	40sec	84.42
G4	500mg	5g	100mg	4.097	40sec	71.94
G5	500mg	5g	150mg	4.973	40sec	89.46
**G6	500mg	7g	150mg	7.118	40sec	94.54
G7	500mg	9g	150mg	7.806	40sec	81.17

nature. In case of G6, the much broader diffraction pattern indicates that the material is amorphous in nature.[8]

Thermal studies

The differential scanning calorimetry thermograms as shown in Figure 3, indicates that the pure GA shows a broad endothermic peak at around 100°C, which may be related with loss of water of crystallization, water bounded with hydrogen as well as remaining water.[18] Also, other endothermic peaks with transition midpoint temperature (Tm) appear in pure GA at 340°C and this may be associated with the thermal degradation in the backbone of the polymer. In case of AM, the thermogram reveals a broad endothermic peak at 75-80°C.[19] But, in case of optimized graft copolymer G6, the endothermic peak with a Tm is at around 347°C. This increase in Tm of the graft copolymer when compared to pure GA, suggests that the graft copolymer is thermally stable to a greater extent than in comparison to pure polymer GA, as the elevation in Tm indicates the thermal stability of any substance.[20]

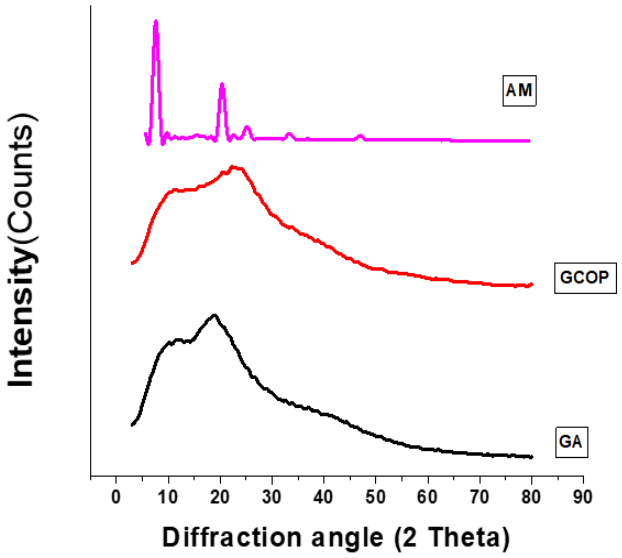


Figure 2: XRD of Pure Gum Acacia (GA), Graft copolymer of Gum Acacia with Acrylamide (G6) and Acrylamide (AM).

Table 2: FTIR spectra with wave number and corresponding functional groups.

Compound	Wave Number (cm^{-1})	Functional Groups
GA	3409	Strong Sharp O-H stretching
	2931	-CH stretching, alkane
	1618	-COO group
	1424	-OH bending , carboxylic acid
AM	3348	-NH stretching
	3201	-NH stretching
	1686	Secondary amide
	1419	-COO- symmetrical vibrations indicating polyacrylamide hydrolysis
G6	3409	Strong broad –OH stretching of Gum Acacia
	1639	Saturated Amide
	1414	Symmetrical -COO- vibrations of the polyacrylamide hydrolysis

Surface morphology of graft copolymer

The surface morphology of pure GA when compared with that of graft copolymer G6 as shown in Figure 4 indicates that grafting has resulted as the surface of the G6 is more rough and wavy, whereas the pure gum particles show smooth edges. Thus, this indicates that grafting reaction has taken place efficiently.[8]

Morphological study of Polymeric scaffold

The SEM images of G6 inserted under the hypodermis of mice skin shows rough surfaces as shown in

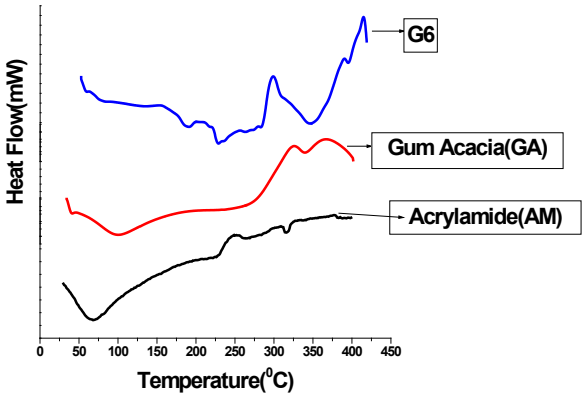


Figure 3: DSC thermograms of Pure Gum Acacia (GA), Graft copolymer of Gum Acacia with Acrylamide (G6) and Acrylamide (AM).

Table 3: C13 NMR spectra.

Compound	Chemical shifts ppm
Physical mixture of Gum acacia (GA) with acrylamide (AM)	169.409
	130.362
	127.913
G6	195.230
	179.653
	170.928
	167.060
	42.503
	39.021
	37.416

Figure 5 A-C, indicates tissue proliferation when compared to that of Figure 5 D (which shows the SEM image of G6 not inserted under the hypodermis). The Figure 5 D, although shows wavy edge and surface, is a clear indication that it is devoid of any cells. These results suggest that optimized formulation G6 is biocompatible with the mammalian cells. Thus, the material G6 can be a very good candidate for tissue growth.[8]

C13 NMR

The C13 NMR peaks of the 1:1 ratio of physical mixture of Gum acacia(GA) combied with acrylamide(AM) and that of optimzed grade graft copolymer G6 are represented in Figure 6a and 6b respectively and in Table 3. The prominent peaks present in the physical mixture as in Figure 6a shows peaks at 169.409 ppm which indicates carbonyl amides and peak at 130.362 ppm and 127.913 ppm indicates the akenes group.Whereas in Figure 6b, there is a shift in the carbonyl amides with more sharp prominent peaks at 195.230 ppm, 179.653 ppm, 170.928 ppm and 167.060ppm indicating that carbonyl amides of AM have attached with GG structure as well as peaks at 42.503 ppm, 39.021ppm and 37.416 ppm indicates presence of alkanes groups. Thus, these results indicates that grafting of AM is successfully done over the GG

Histological investigations for Polymeric scaffold

For detection of toxicity, the optimized grade G6 (GA-g-AM) after insertion under the mice hypodermis, local tissue was investigated for both control as well as the test animal (Figure 7). The test tissue section showed significant evidence of fibroblasts (F) as well as very dense collagen growth (C). These results indicates that the presence of material G6 promotes cell proliferation faster when compared with that of the control tissue and also completely biocompatible and can be utilized as polymeric scaffold for tissue proliferation.[21]

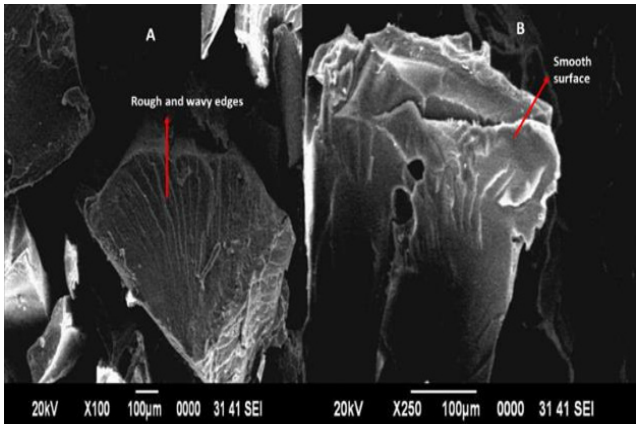


Figure 4: SEM images of A (Graft copolymer G6) and B (Pure Gum acacia, GA).

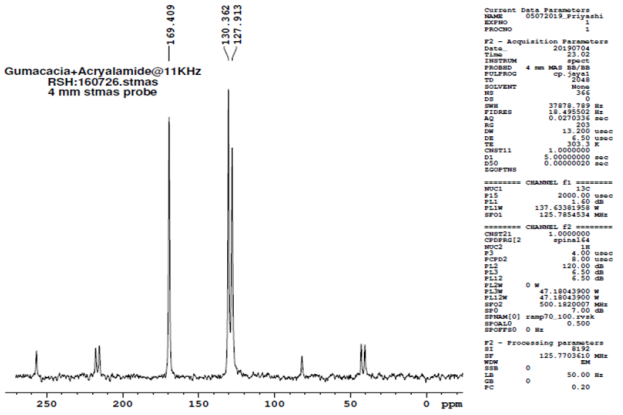


Figure 6(a): C13 NMR spectra of Physical mixture of Gum acacia with acrylamide.

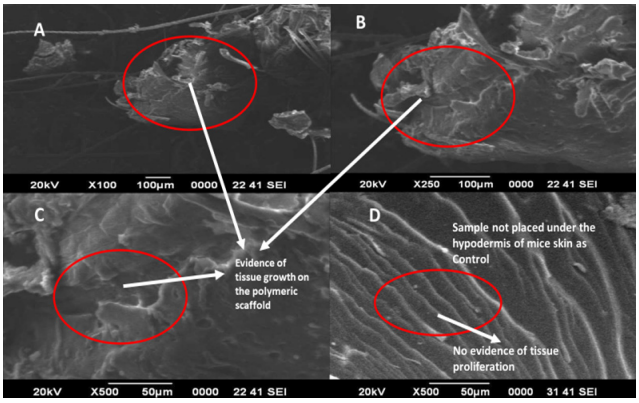


Figure 5: SEM images of tissue growth on G6 polymeric scaffold (A,B,C), SEM image of G6 not placed under the hypodermis as Control(D).

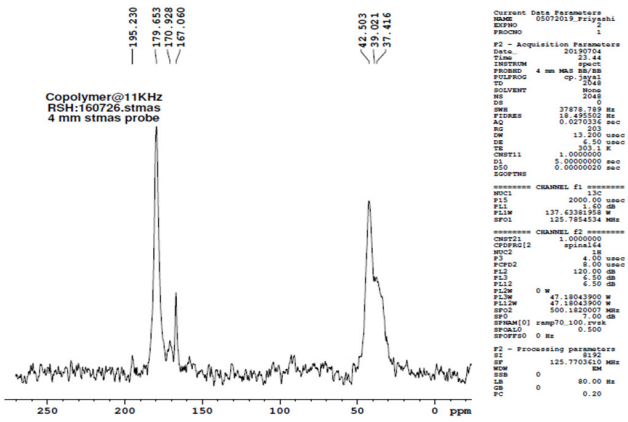


Figure 6(b): C13 NMR spectra of optimized graft copolymer grade G6.

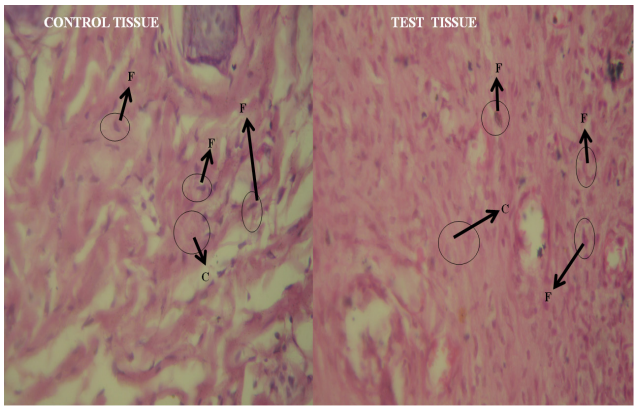
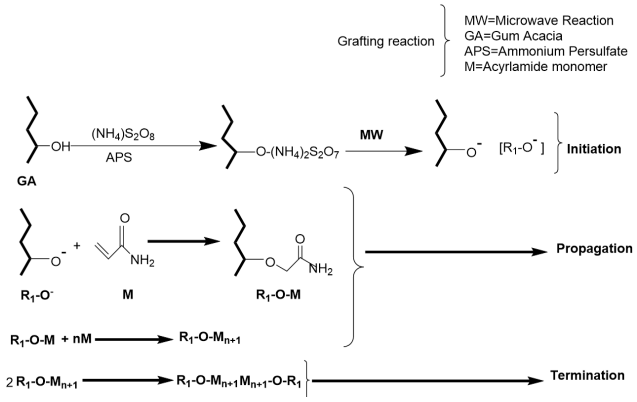


Figure 7: Histopathological view of normal skin and skin inserted with G6 (GA-g-AM) on 8th day after surgery (A. Control; B. Test mice). Skin section showed hematoxylin and eosin stained epidermis and dermis by utilizing Leica Microscope at 40X respectively where F&C shows spindle shaped fibroblast and collagen respectively.



Scheme 1: Reaction scheme of graft copolymer of Gum acacia grafted with acrylamide (GA-g-PAM), where in the Initiation step, the main polymer backbone Gum acacia reacts with the redox initiator ammonium per sulfate (APS), to form a free radical site onto the polymeric backbone. And with the addition of monomer acrylamide (AM), it starts to attach to the free radical site and starts polymerization in the Propagation step, where the monomer starts forming chains and later the reaction is terminated by ice cold water and acetone in the Termination step

CONCLUSION

Gum acacia-g-acrylamide (GA-g-PAM) was effectively prepared by microwave assisted synthesis utilizing ammonium persulfate as the redox initiator. Microwave irradiation procedure is utilized based on the fact that it yields higher percentage grafting efficiency and better reproducibility. The optimized graft copolymer G6 was taken as the best grade based on the optimum content of APS and AM. The different analytical characterizations using FTIR, DSC and XRD studies confirmed the grafting of the monomer AM on the polymer (GA) backbone and SEM studies along with the histological studies indicates the tissue proliferation capability of the graft copolymer G6. Thus, it may be suggested that the optimized grade G6 can be a versatile material for tissue replacement as polymeric scaffold.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Author's Contribution

The experimental study was done by all the co-authors and the corresponding author Trishna Bal designed the experiment and drafted the manuscript and finalized the article. All authors read and approved the final copy of the text.

ABBREVIATIONS

APS: Ammonium Persulfate; **%GE:** % Grafting efficiency; **GA:** Gum acacia; **AM:** Acrylamide; **FTIR:** Fourier Transform Infrared spectroscopy; **DSC:** Differential Scanning calorimeter; **XRD:** Xray diffraction; **NMR:** Nuclear magnetic resonance spectroscopy; **SEM:** Scanning electron microscopy; **PAM:** Polyacrylamide; **GA-g-PAM:** Polyacrylamide grafted gum acacia; **G6:** Sample name; **CIF:** Central Instrument facility.

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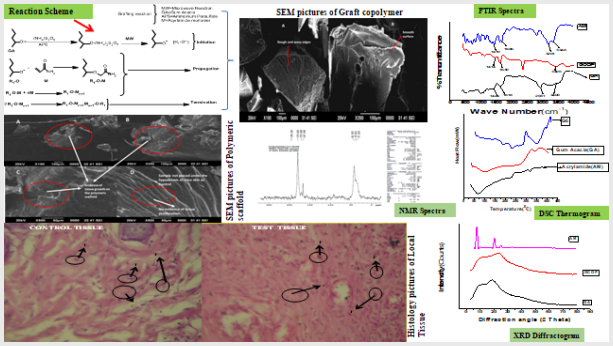
PICTORIAL ABSTRACT



About Authors

Prof. Trishna Bal, Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, INDIA.

SUMMARY

The use of natural polymers as scaffolds for cell proliferation are advantageous as these polymers are nontoxic and almost mimic the extracellular matrix. Moreover, these polymers are very cheap and easily available being abundant in nature. But natural polymers lack in being stable and thus to enhance their stability, modifications with synthetic polymers or monomers are done. Thus the present research is based on preparation and application of Polyacrylamide grafted Gum acacia graft copolymer (GA-g-PAM) as polymeric scaffold when inserted under the skin hypodermis of mice. The preparation of the graft copolymer was done using microwave irradiation along with redox initiator for initiating free radicals thereby polymerization to complete. The optimization of the best grade was done by percentage grafting efficiency (%GE) along with the analytical characterizations of other grades using FTIR, DSC, XRD and C13 NMR. The histopathology slide of local tissue where the polymer sample was applied evidenced good collagen growth and fibroblast cells as compared to that of control, proving that the material is biocompatible and can be utilized as polymeric scaffold in wound healing.

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