ISSN 0974-4169 (Print)
0974-4150 (Online)

www.ajrconline.org

RESEARCH ARTICLE

Synthesis, Characterisation, Biological and Molecular Docking Studies of Aconitic Acid Based Co-Polyester

Vanitha Krishna Kumar, Mrs. Nanthini Raveendiran
Postgraduate and Research Department of Chemistry, Pachaiyappa's College, University of Madras, Chennai, India.
*Corresponding Author E-mail: **vanabhava@yahoo.co.in**

ABSTRACT:
Aliphatic polyester elastomers are class of biomaterials that are widely used for drug delivery and tissue engineering. The demand for biodegradable non toxic elastomers is ever increasing due to their dexterity of being biocompatible and biodegradable. A series of novel biodegradable polyesters are synthesised, using multifunctional non- toxic monomers: aconitic acid, citric acid, sebacic acid and glycerol by melt condensation method, followed by post polymerisation. The chemical structure of the elastomer are characterised by FT-IR, ^{1}H NMR and ^{13}C NMR.TGA, and DSC techniques are used to determine the physical properties of the polymers. The low Tg values and a higher Tm and Td illustrates the elasticity and stability of the synthesised elastomers respectively which makes it suitableas implant materials.The CAM assay exhibits the wound healing property which is further confirmed by the molecular docking with the matrix metalloproteinase. Chorioallantoic membrane (CAM) assay shows that the formation of blood vessels is 178% and 169% greater than the standard. Lib dock score of these polymers with MMP2, MMP8and MMP12 suggests that are effective MMP inhibitors. The observed results unambiguously suggest that the synthesized elastomer poly(glycerol acotinate-co-glycerol citrate)PACGL and poly(glycerol acotinate-co-glycerol sebacate)PASGL have excellent wound healing ability and are suitable as potent biomaterials in the biomedical field.

KEYWORDS: Elastomer, Aconitic acid, Molecular docking, CAM assay, Implant biomaterial.

INTRODUCTION:
Unsaturated aliphatic polyester elastomers have gained a lot of attention as an implant material in the field of tissue engineering due to their biocompatibility and biodegradability. Numerous elastomers are being explored, via synthesis and application, yet the quests for novel biodegradable elastomers are on rise, to meet the ever increasing demand. Unfortunately, implantation of many such polymers have resulted in adverse effects such as inflammation, low cell adhesion etc and also required a complex and expensive synthetic procedure[1,2]. Elastomers using caprolocatone[3], citric acid[4], sebacic acid [4,5] and Aconitic acid[7,8] has been reported in the past. Yet, no study has been reported based on co-polymerisation of aconitic acid and citric or sebacic acid with glycerol.

The present work is aimed at synthesising biocompatible, biodegradable, random copolyester elastomers, based on multifunctional as well as non toxic monomers; Aconitic acid, citric acid, sebacic acid and glycerol without the use of any toxic catalyst. The unique monomers are extracted from sugarcane baggase and from natural components unlike other monomers which are petroleum based. Moreover aconitic acid and citric acid are also constituent metabolites in the Kreb's cycle. Even though these metabolite monomers are not essential to synthesise biocompatible elastomers, but it attempts a route to minimize the side effects as these molecules are resorbed in various physiological pathways during metabolism[9].

Received on 22.03.2018 Modified on 14.04.2018
Accepted on 26.04.2018
Asian J. Research Chem. 2018; 11(4):723-730.
DOI: 10.5958/0974-4150.2018.00128.1

Poly(glycerol acotinate-co-glycerol citrate) PACGL and poly(glycerol acotinate-co-glycerol sebacate) PASGL were synthesized, characterized and subjected to biological studies such as CAM assay[10,11] to establish their wound healing ability and biocompatibility. The molecular interaction of synthesised polymers with matrix metalloproteinase using molecular docking studies displayed wound healing property of the elastomers, which is illustrated by the inhibition of matrix metalloproteinase[12-15].

MATERIALS AND METHODS:

Synthesis of elastomers:

These polyesters were prepared in simple two step methods of synthesising pre-polymers by melt poly-condensation method without the use of a catalyst followed by post-polymerisation. Equimolar amounts of monomers were placed in a three necked flask and heated up to 170ºC-175ºC for 15 minutes, till monomers melts. The temperature is then decreased to 140ºC-145ºC for 2 hours in nitrogen stream. The purified multifunctional pre-polymers are dissolved in 1,4-dioxane[6] and casted into a silicone petri dish, left overnight and then placed in an air oven for about 24hrs at a temperature of 80ºC. The post polymers were obtained in the form of thin film.

FT-IR, ^{1}H NMR, ^{13}C NMR and MALDI-MS were performed using the pre-polymers, due to the insolubility of the cross-linked post polymers. Thermal and biological studies were performed using the post polymers.

Polymer characterisation:

A Fourier transform infra red spectrum was recorded using FT-IR 4700 type A spectrometer for the pre-polymer samples prepared by a solution casting technique over KBr crystals.^{1}H NMR and ^{13}C NMR were recorded for the purified pre-polymers, dissolved in DMSO using 400MHz solution Bruker Flex-PC microflex NMR spectrometer. The molecular weight of the pre-polymers were determined by BRUKER 1825 MALDI mass spectrometer using the pre-polymers dissolved in DMSO with the ionising agent NaI.

Thermogravimetric analysis(TGA) of the post polymers were performed in a range of room temperature to $500^{0}C$ in nitrogen atmosphere at a heating rate of $10^{0}C$/min with a TGA instrument TGA Q50 V20.13 Build79. Differential scanning calorimetry (DSC) was performed in a range of $-70^{0}C$ to $350^{0}C$ at a heating rate of $10^{0}C$/min using DSC Q200 V23 Build 79 instrument under nitrogen atmosphere.

Biological studies:

CAM assay (*Chorion allaontoic membrane)* was used to find the biocompatability of the synthesised post polymers. Synthesized compounds were dissolved in sterile phosphate buffer saline and pellets of these solutions 100 µL/pellet (Corresponds to 1000 µg/pellet) were prepared, applied drop wise on gelatine sponge and then on to chorioallantoic membrane. Seven day embryonated white leghorn chicken eggs were cleaned with 70% ethanol and asmall window of 1.0 cm^{-2} cm was made in the shell of each egg. Then, air was sucked out from the eggs to bring their membrane down[11]. Through the window of each egg, sterile disc of gelatine sponge containing concentration (1000 µg/pellet) of methanol extracts were implanted inside the egg at the junction of two blood vessel of the chorioallontoic membrane. Subsequently, the opening was resealed with parafilm and the eggs were re-incubated at 37º C and incubated for 72 hrs[10]. The windows were then reopened and formation/inhibition of vessels were observed in terms of number and caliber and finally compared with PBS used as control.

Molecular docking studies was performed using Acclery's Discovery studio 4.0 in TANUVAS, Chennai to identify the wound healing property of the synthesised polyesters. Three dimensional structures of three different matrix metalloproteinase MMP2 (gelatinase), MMP8 (collagenases) and MMP12 (metallo elastase): (PDB ID:1QIB;1MNC; 1HNE) were obtained from the protein data bank[12]. The structures of PACGL and PASGL were drawn using CHEMBIODRAW and converted in the form of sdf format. The prepared ligands were used as input files for Auto docking. Studio Visualizer and AutoDock Tools packages were used to prepare docking files. A grid of 60, 60, and 60 points in *x*, *y*, and *z* directions was built with a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map. The default settings were used for all other parameters. Lamarckian genetic algorithm method was employed for docking simulations [13]. At the end of docking, the best poses were analyzed and the pose with the highest lib dock score was considered.

RESULTS AND DISCUSSION:

Polymer Characterisation:

The FT-IR spectra (Fig1 and Fig2) shows strong absorption bands at 1713cm^{-1} and 1725cm^{-1}, which contributes to the carbonyl stretching of the ester bond. Moreover the ester bond formation is confirmed by the strong absorption at 1160 cm^{-1} in both the spectra, which due to the C-O stretching of the ester group. The peaks at 3652 cm^{-1} and 3349 cm^{-1} are attributed to the -OH stretching of the glycerol moiety. The absorption band at 2949 cm^{-1} were assigned to the methylene groups in the triol and acids[4].

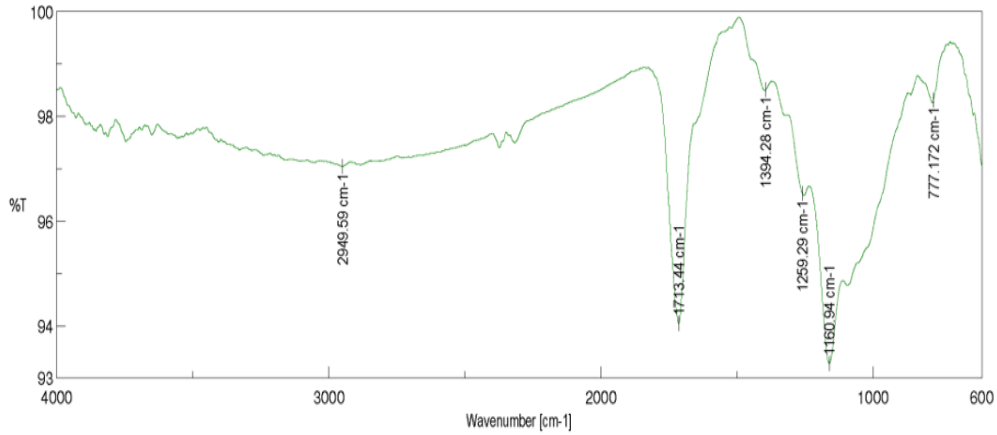


Fig1 FT-IR spectra of PACGL

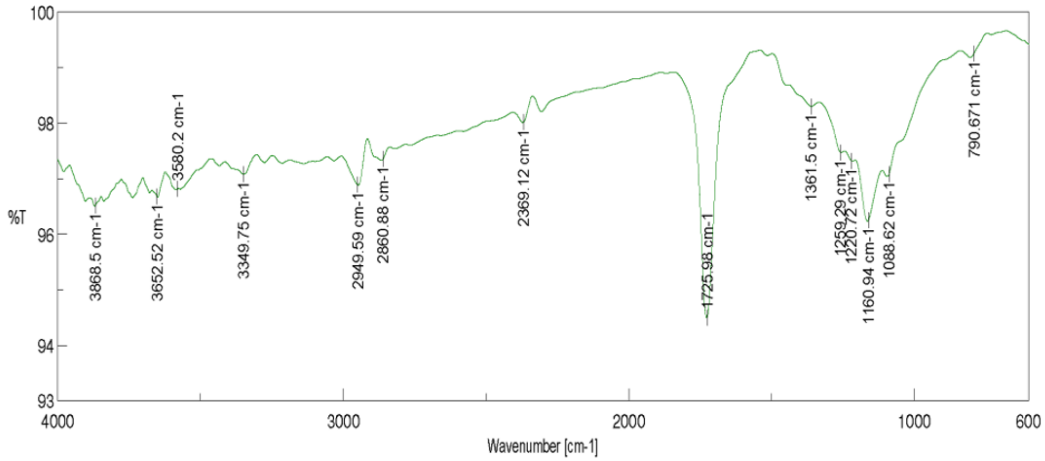


Fig2 FT-IR spectra of PASGL

The ^{1}H NMR spectra of the pre-polymers (Fig:3 and Fig:4) show peaks aroundδ 3.5- δ 3.7 ppm due to the –$CH_2$and –CH groups ofglycerol moiety. The peaks at δ 6.76 ppm andδ 6.8ppm are due to the –CH group of Trans Aconitic acid. The δ 2.8ppmof PACGL (Fig:1) is attributed to the –CH_2 group of citric acidand δ 0.9ppm - δ 1.2ppm is of PASGL (Fig:2) is attributed to the-CH_2 group of sebacic acid. The ^{13}C NMR of these pre-polymers PACGL (Fig:5) and PASGL (Fig:6) shows peaks at δ160-180ppm which confirms the formation of the ester group.

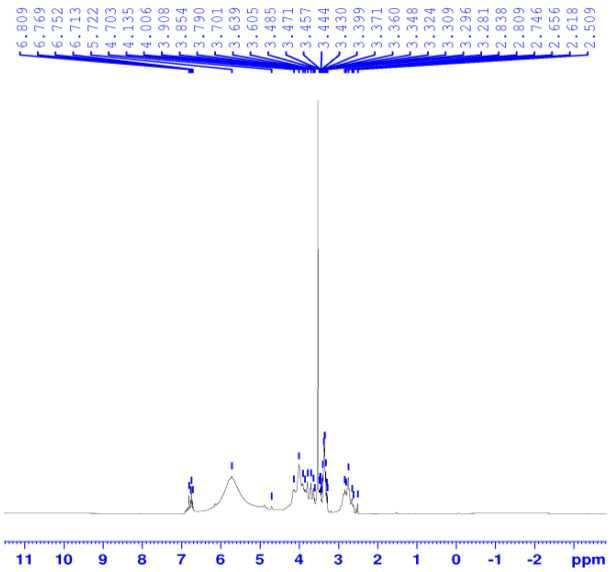


Fig:3 ^{1}H NMR spectra of PACGL

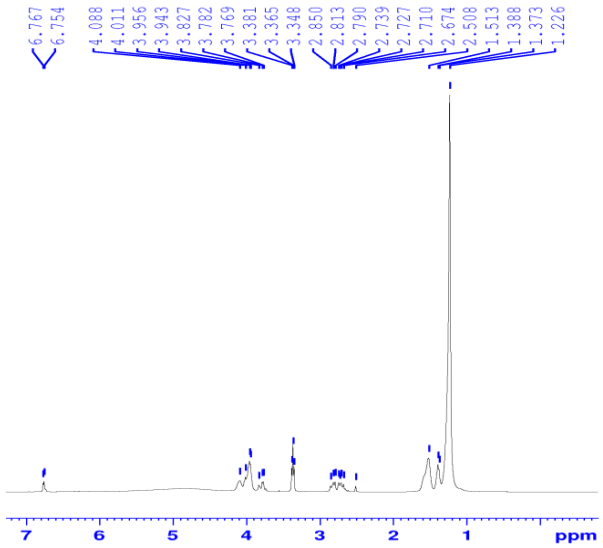


Fig:4 ^{1}H NMR spectra of PASGL

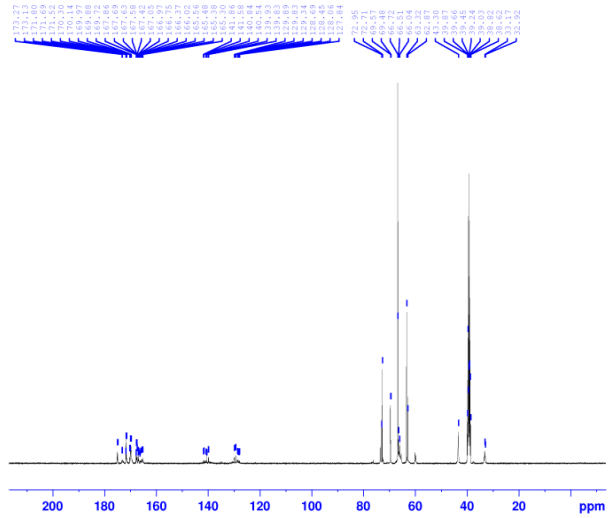


Fig:5 ^{13}C NMR spectra of PACGL

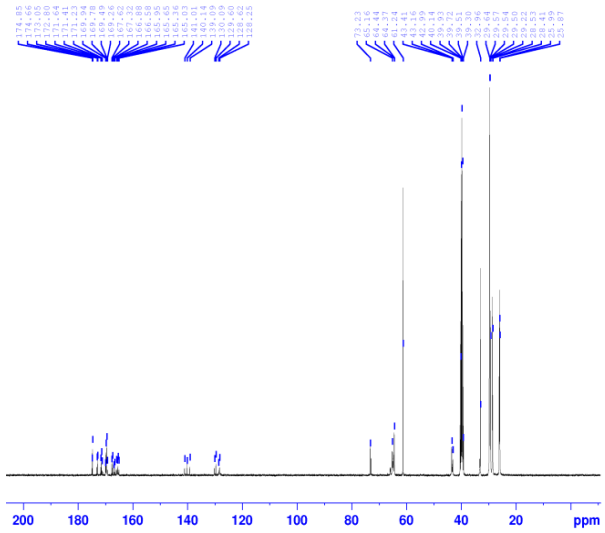


Fig:6 ^{13}C NMR spectra of PASGL

The MALDI-MS spectrum of the pre-polymer PACGL shows the molecular ion peak at 679, which is assignable to (TA_2GL_2CANa). The m/e of PASGL at 925 is assigned to the fragment ($TA_2GL_3SA_2Na$). The mass spectrum confirms the formation of the oligomers.

The DSC and TGA values are listed in the table: 1. The thermal studies reveal the cross linking and the elastomeric properties of the post polymers by the lower T_g value of -17.38ºC and -64.54ºC for PACGL and PASGL respectively as shown in fig 7 and fig 8. The high decomposition temperature T_d (Fig 9 and fig 10)displays the thermal stability of the post polymers. The melting point T_m, muchabove the body temperatureillustrates its suitability as implant materials. Comparatively a very low T_g value for the polymer PASGL is attributed to the Chain length of sebacic acid than the citric acid. The lower Tg value attributes to the elastic nature of the polymers which is a unique quality of implant materials.

Table:1 Tg, T_m, T_d, valuesof PACGL and PASGL

Elastomer	T_g/ ºC	T_m/ ºC	T_d/ ºC
PACGL	**-17.38**	**157.22**	**288.63**
PASGL	**-64.54**	**84.94**	**323.70**

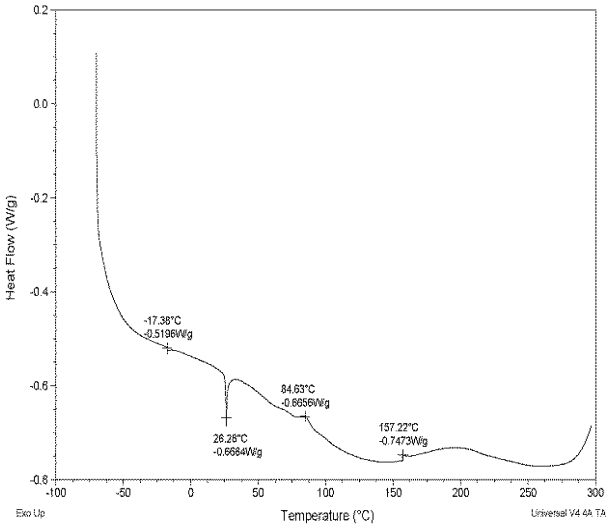


Fig : 7 DSC thermogram of PACGL

Table : 2 No. of Binding poses , Lib dock score and relative energy values of PACGL and PASGL

Elastomer	*Type of MMPs*	*No. of binding poses*	*Lib dock score*	*Relative energy/ KJ/Mol*
PACGL	MMP-2	13	86.68	2.2532
PACGL	MMP-8	16	97.73	7.0211
PACGL	MMP-12	17	83.16	3.7497
PASGL	MMP-2	21	98.48	3.2153
PASGL	MMP-8	20	110.00	5.1055
PASGL	MMP-12	15	92.93	4.7657

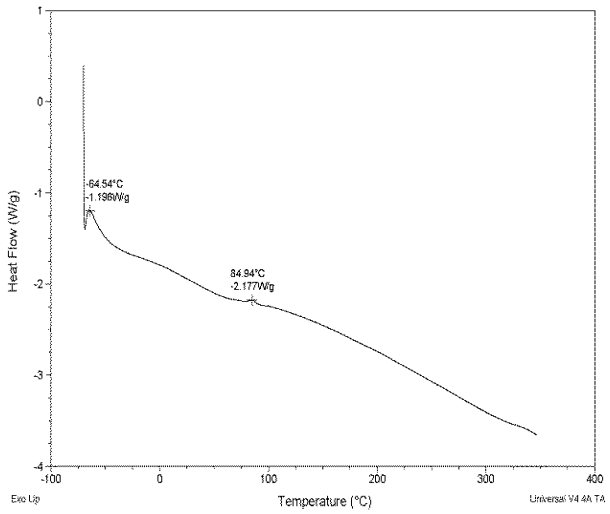


Fig : 8 DSC thermogram of PASGL

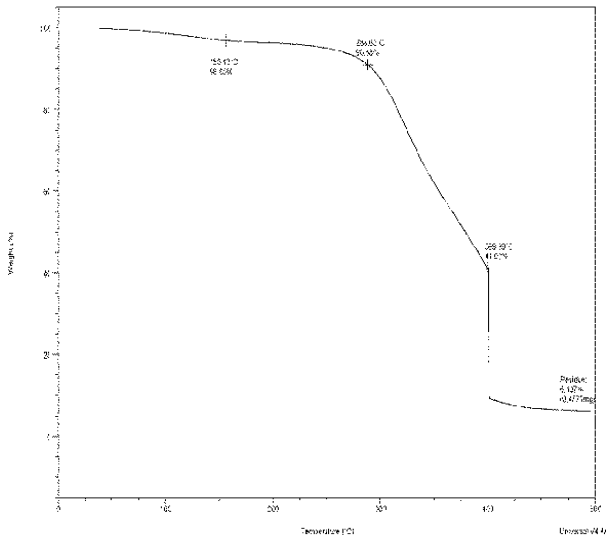


Fig : 9 TGA thermogram of PACGL

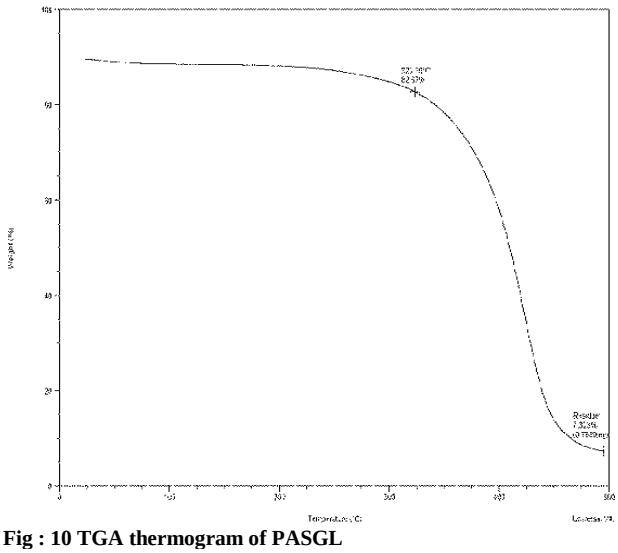


Fig : 10 TGA thermogram of PASGL

Biological studies:

CAM Assay:

The CAM assay confirms the biocompatibility of PASGL, as it can form blood vessels 169% greater than the control.The number of branch point of the blood vessels formed by PACGL 178% is greater than the control. The PACGL is more biocompatible due to the presence of citric acid moiety. The following figures shows the blood vessels formed by the control(Fig:11), PASGL(Fig:12) and the PACGL(Fig: 13)respectively.

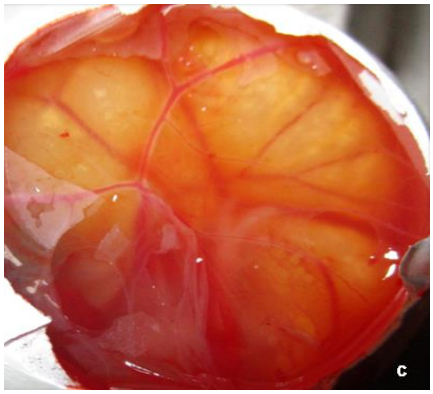


Fig:11 CAM assay of the control showing the formation of blood vessels

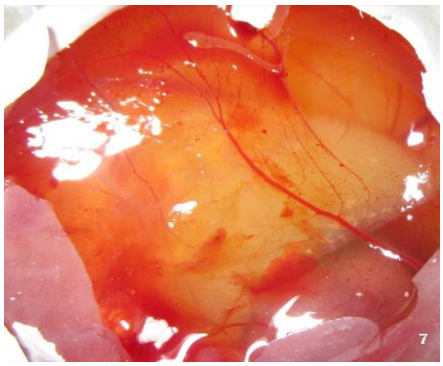


Fig:12 CAM assay of the PASGL showing

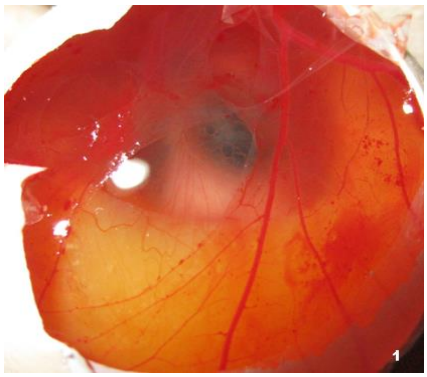


Fig:13 CAM assay of the PACGL showing the formation of blood vessels the formation of blood vessels

The CAM assay shows the formation of the new blood vessels, which supports the wound healing process after the implantation of the biomaterial. After 72 hrs, endothelial cells became adherent and showed confluent cell layers formed on the synthesised polymer and also on the window of the egg[10-11].

This result shows the biocompatibility of the polymer to well adhere to the surface of the implanted site and also to mimic the ECM. The biocompatibility of these polymers is due to natural monomers used, whichis evident from the growth of the blood vessels , which can be owed to the natural monomers as they are intermediates in the Kreb's cycle.

Molecular docking studies:

Matrix metallo proteinase plays essential and beneficial roles in three important phases of wound healing like inflammation, proliferation and remodelling. They play key roles in debriding damaged ECM, angiogenesis, re-epithelialisation, wound contraction, and scar remodelling[14,15]. However, there is strong clinical evidence that chronically elevated levels of MMPs and other proteases prevent wounds from healing [16,17].In acute wounds, the increased activity ofMMP's causes, ECM and basement membrane destruction in excess which contributes to the pathogenesis of many diseases.It is thus desirable to reduce or inhibit the effects or activity of MMPs in a patient suffering from a variety of diseases, conditions, and disorders. It has been found that many polymers and polymer compositions are effective inhibitors of MMPs.

The molecular docking studies is used to find the inhibiting activity of the synthesized polymers to avoid the undesired activity of MMPs (MMP-2, MMP-8 and MMP-12). The studies revealed that PASGL and PACGL reduces or inhibits the unwanted or elevated MMPs, activity.

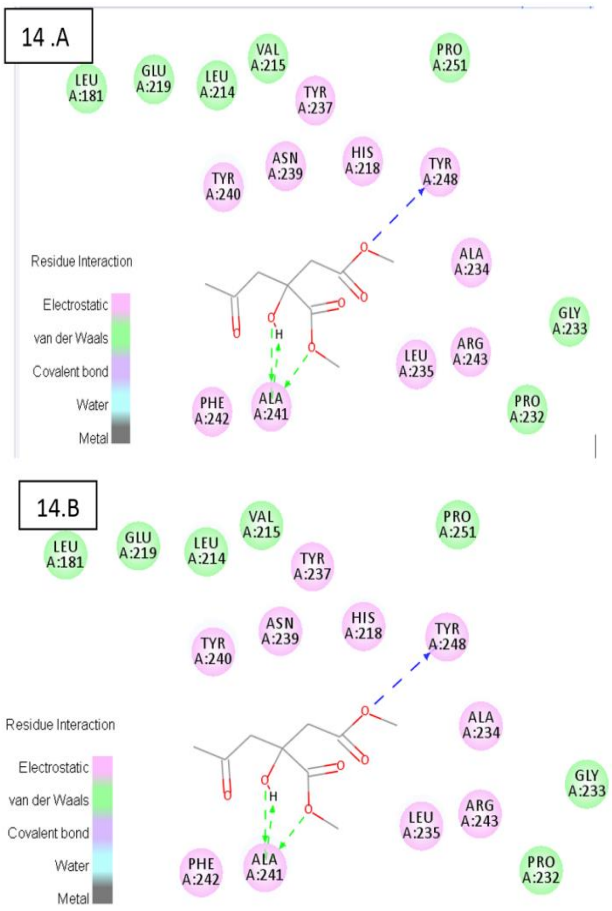
Molecular docking studies reveal that the elastomers PASGL and PACGL docked into the active sites of MMP-2, MMP-8 and MMP-12. The literature [18] suggests that if the RMSD of the best docked structure is $\leq$ 2.0A^o then the scoring function is successful. The RMSD value of the elastomers is less than 2.0 A^o, which further confirms the reliability of docking of the synthesised polymers into the structures of MMPs. Moreover the greater the Libdock score values and lower the relative energy values, stronger is the affinity between the ligands and the MMPS. The table:2list out the relative energies and Libdock score values of the best poses of the synthesised elastomers with MMP-2. MMP-8and MMP-12.

Table : 2 No. of Binding poses , Lib dock score and relative energy values of PACGL and PASGL

Elastomer	*Type of MMPs*	*No. of binding poses*	*Lib dock score*	*Relative energy/ KJ/Mol*
PACGL	MMP-2	13	86.68	2.2532
PACGL	MMP-8	16	97.73	7.0211
PACGL	MMP-12	17	83.16	3.7497
PASGL	MMP-2	21	98.48	3.2153
PASGL	MMP-8	20	110.00	5.1055
PASGL	MMP-12	15	92.93	4.7657

These values suggest that both the elastomers have good inhibitory potential against MMPs[19]. Comparatively PACGL has better libdock score which can be due to the presence of citric acid moiety. The binding mode of these ligands with the active site of the MMPs is shown using the 2D ligand- protein interaction. The wound healing property of these elastomers is visible from their amino acids interactions as shown in the figure.

PACGL has a binding interaction with Ala A:220with MMP2(Fig:14 A). The potential binding site of PACGL with MMP-8 is found at Ala A:241 and TYR A:248 (Fig:14 B).The binding interactions of PACGL with MMP-12 was at Leu E:130, Cys E:182 and Arg E:129(Fig:14 C).



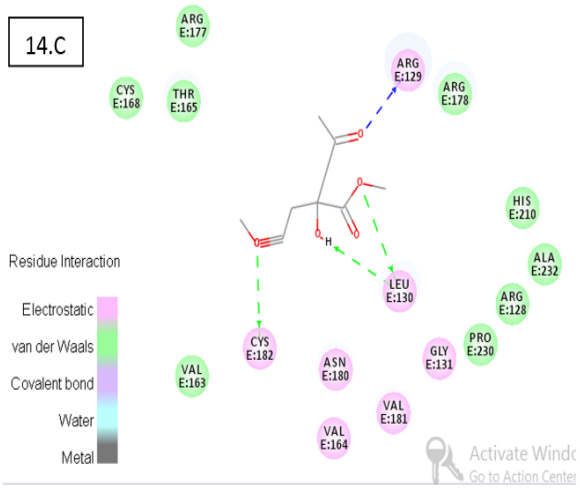


Fig:14 Binding interactions of amino acids with PACGL

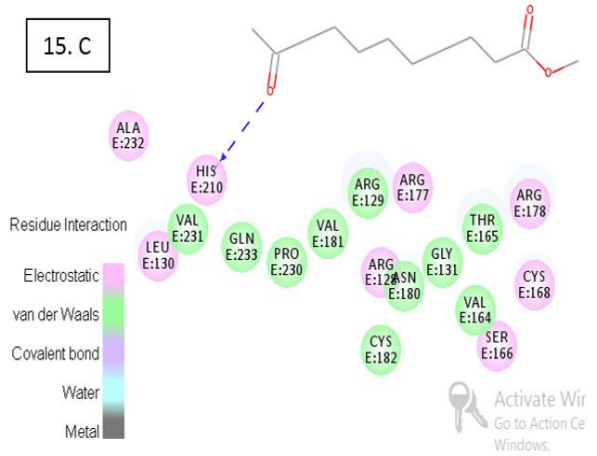
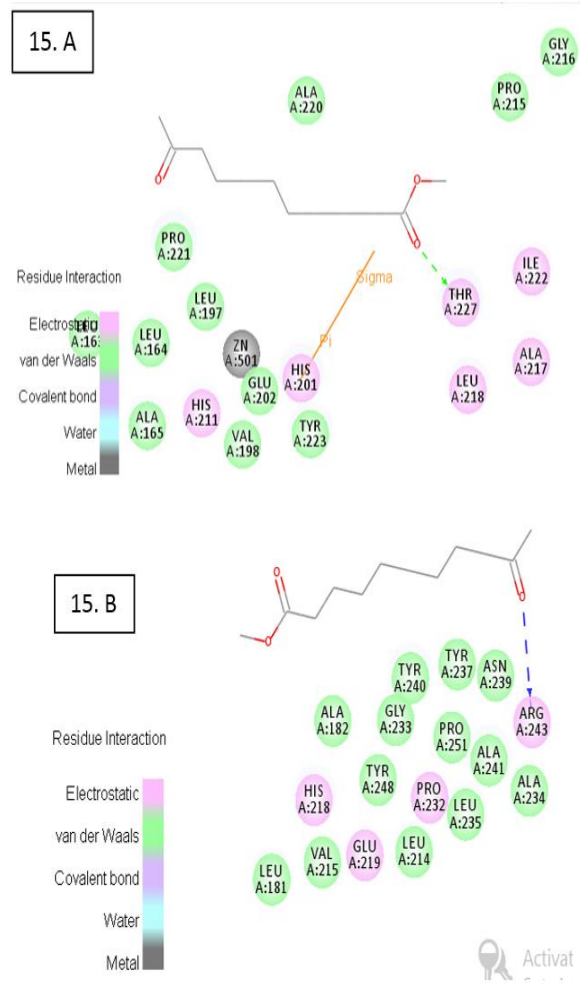


Fig: 15 Binding interactions of amino acids with PASGL

The binding interactions of PASGL with MMP-2 is found at His A: 201 and Thr A:227(Fig:15 A). PASGL has a hydrogen bonding interaction at Arg A:243 with the MMP-8 proteinase (Fig:15 B). The binding site of PASGL with MMP-12 is found at His E:210 (Fig:15 C).



The MMP protease inhibiting property of the synthesised elastomers PASGL and PACGL is evident from the hydrophilic and hydrophobic interaction of the elastomers with the amino acid residues present in the active sites of the MMPs. This affirms that the synthesised elastomers possess the wound healing ability, which makes it suitable as an biomaterial

CONCLUSION:

The polyester elastomers Poly(glycerol acotinate-co-glycerol citrate) PACGL and poly(glycerol acotinate-co-glycerol sebacate) PASGL were synthesized by simple melt poly condensation methods without the use of any toxic catalyst.PASGL and PACGL were characterized by FT-IR, NMR spectroscopy and MALDI-MS. The cross-linking in the synthesised elastomers is evident from the low glass transition temperature. The melting point and decomposition temperature displays the thermal stability of the elastomers on implantation, as their decomposition temperature was much above the body temperature. The biocompatibility of the elastomers is obvious from its ability to form blood vessels from the CAM assay. Molecular docking studies divulge the MMP inhibiting potential of the elastomers by the interactions with the amino acid residues in the active site of the MMPs. These results confirm the elastomers PACGL and PASGL have the potential as an implant biomaterial in the field of tissue engineering.

ACKNOWLEDGEMENT:

The author would like to thank CATERS, CLRI Chennai for their analytical support and Bio- Informatics, TANUVAS, Chennai for the molecular docking studies.

CONFLICT OF INTEREST:

The author declares no conflict of interest.

REFERENCES:

1. Richard T. Tran, Jian Yang and Guillermo A. Ameer. Citrate based biomaterials and their applications in regenerative medicine. Annu: Rev Materials. 2015; 45:277- 310.
2. Pathiraja A. Gunatillake and Raju Adhikari. Biodegradable synthetic polymers for tissue engineering European Cells and Materials. 2003; 5: 1-16.
3. Younes HM, Bravo-Grimaldo E and Amsden BG. Synthesis, characterization and in vitro degradation of a biodegradable elastomer. Biomaterials 2004; 25(22), 5261-5269.
4. J Yang et al.Synthesis and Evaluation of Poly(diol Citrate) Biodegradable elastomers. Elsevier- Biomaterials 2006; 27 ,1889–1898.
5. Simitzis J et al. Synthesis and characterization of hydrolytically degradable copolyester biomaterials based on glycolic acid, sebacic acid and ethylene glycol. Journal of Material Science: Materials in Medicine. 2012; 22(12):2673-2684.
6. Djordjevic et al. Synthesis and characterisation of novel citric acid based polyester elastomers. Polymer 2009; 50 :1682-1691.
7. Hongliang Cao and Yu Zheng. A novel hyperbranched polyester made from aconitic acid (B3) and di(ethylene glycol) (A2). Polymer International 2010; 60(4):630-634.
8. Akanksha Kanitkar, Mollie Smoak, Cong Chen, Giovonna Aita, Thomas Scherr, Lee Madsen and Daniel Hayes. Synthesis of novel polyester for potential application in skin tissue engineering. Journal of Chemical technology and biotechnology 2017; 91(3): 563–836.
9. Devin G. Barrett and Muhammad N. Yousaf. Design and Applications of Biodegradable Polyester Tissue Scaffolds Based on Endogenous Monomers Found in Human Metabolism. Molecules 2009 ;14(10): 4022-4050
10. Yeh WC et al. FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science, 1998; 279(5358):1954-1958
11. Lobb et al. Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. Biochemistry. 1985; 24(20):5480-5486.
12. Gurudeeban Selvaraj*, Satyavani Kaliamurthi and Ramanathan Thiruganasambandam. Molecular docking studies of rutin on matrix metalloproteinase. Insights in Biomedicine, 2016; 1:1-4.
13. Musa A. Ahmed et al. Structure-based design, synthesis, molecular docking, and biological activities of 2-(3-benzoylphenyl) propanoic acid derivatives as dual mechanism drugs. Journal of Pharmaceutical Bioallied Science. 2012; 4(1): 43–50.
14. Gibson D et al. MMPs made easy. Wound healing. 2009; 1(1):1-6.
15. Sean E. Gill* and William C. Parks. Metalloproteinase and Their Inhibitors: Regulators of Wound Healing.International Journal of Biochemistry and Cell Biology. 2008; 40(6-7): 1334–1347.
16. Veves A, Sheehan P, Pham HT. A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. Arch Surg 2002; 137(7): 822-27.
17. Lobmann R, Zemlin C, Motzkau M, et al. Expression of matrix metalloproteinases and growth factors in diabetic foot wounds treated with a protease absorbent dressing. Journal of Diabetes Complications 2006; 20(5): 329-3
18. Paul MK, Mukhopadhyay AK. Tyrosine kinase-role and significance in cancer. International Journal of Medical Science, 2004; 1:101–15
19. Talambedu Usha et al. Molecular docking studies of anti-cancerous candidates in *Hippophaer hamnoides* and *Hippophae salicifoli*. J Biomed Res. 2014;28(5):406–415.