

RESEARCH ARTICLE

Study on the inflammatory response of PMMA/polystyrene/silica nanocomposite membranes for drug delivery and dental applications

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Abstract

Background

The application of polymeric materials in medical industry has grown drastically in the last two decades due to their various advantages compared to existing materials. The present research work emphasizes on the sol-gel technique to formulate the polymethyl methyl acrylate/polystyrene/silica composite membrane.

Methods

The characteristic of the composite was investigated through modern state art of instrumentation.

Results

The functional groups attached to the polymer was absorbed by FTIR. The FTIR spectrum confirm that the blend was mixed thoroughly and the formation of unite intimately between the polymers. The membranes were observed by SEM for its surface homogeneity which depends upon the composition of the two blending polymers. The captured SEM images showed the formation of microcracks on the surface, which was evidently controlled by varying the constituent polymer ratios. The prepared blend membranes with 2:1 ratio of PMMA/PS/Si displayed higher water uptake compared to other blended membranes. The composite membranes had good hydroxyl apatite growth in SBF solution. Furthermore, the *in vitro* cytotoxicity studies carried out by MTT method, using RAW macrophage cells showed that all the samples exhibited excellent cell viability.

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Conclusion

The inflammatory response of composite with equal concentration of PMMA-PS were performed and observed no inflammation in comparison with control and other tested concentrations.

Introduction

Immense research in biomaterials used for hard and soft body tissues replacement and orthopedic applications were constantly increasing during the past few decades[1–5]. Biomaterials, used for this kind of replacement should be inert, bioactive and biocompatible. Based on the type of implant needed, the type of material like metals, alloys, ceramics and polymeric materials can be selected as a suitable biomaterial [6]. Among various kinds of biomaterials, polymeric biomaterials have gained more importance in recent days due to its vast advantages. Moreover, polymeric biomaterials are being used as a replacement of metallic materials (amalgam) due to their added advantage like light weight and tailor made properties[6]. There are several natural polymers like chitosan, cellulose having bioactivity and biocompatibility are used as biomaterials for various wide range of biomedical applications[7, 8]. However, the applications of these natural polymers are limited in terms of its stability and strength. The synthetic polymer is an alternative to natural polymers which may enhance the stability, strength and biocompatibility. These tailor-made properties of synthetic polymers improve its medical and biomedical applications. There is list of synthetic polymers such as polyamides (PA), polyethylene (PE), polyether ether ketone (PEEK), poly methyl methacrylate (PMMA), polysulfone (PSu), polytetrafluoroethylene (PTFE), polyurethane (PU), and ultra-high molecular weight polyethylene (UHMWPE) used as biomaterials with inorganic nano-bio-materials to induce bioactivity. The bioactivity of these polymers were induced by functionalizing the polymer or by blending with other polymer having bioactivity[9].

The PMMA is the most successful and investigated material in medical application such as implant in orthopedic applications due to its good bioactive and biocompatibility nature when used as bone cements in hard tissue replacements. Despite the many drawbacks like brittleness, shrinkage and high polymerization exotherm it used as bone cement for orthopedic applications is still serves a greater advantage. Similarly, polystyrene is one of the highly researched topic in biomedical applications known for its inert nature and has its applications in consumer, food packing industries has shown a good bioactivity and enhanced cell adhesion when modified with silica [10, 11]. It is an important criterion to use porous bioactive polymer matrix since it is more advantageous considering the ability of hydroxyapatite to grow within the pores, this phenomenon affixes the formation of interlock with the adjacent normal bone thereby lifts the fixation of polymer prosthesis. The application of these polymer matrix as biomaterials greatly influences its biological and mechanical properties in internal bone implants due to the tailor-made nature [12]. Regardless of advantages of PMMA possess a major drawback that is causing inflammation after the removal of the prosthesis from the implant site [13–14] will be addressed in the present study.

The sol-gel technique has been successfully used for preparation of PMMA/Polystyrene/Silica nanocomposite membrane. The sol-gel technique is selected due to various advantages includes, commendable chemical homogeneity, controlled size and morphology, operates at low temperature [15–17]. The sol-gel composed membranes are highly embodied matrices, which are used in the fields of biomedical sensors, laser materials and for

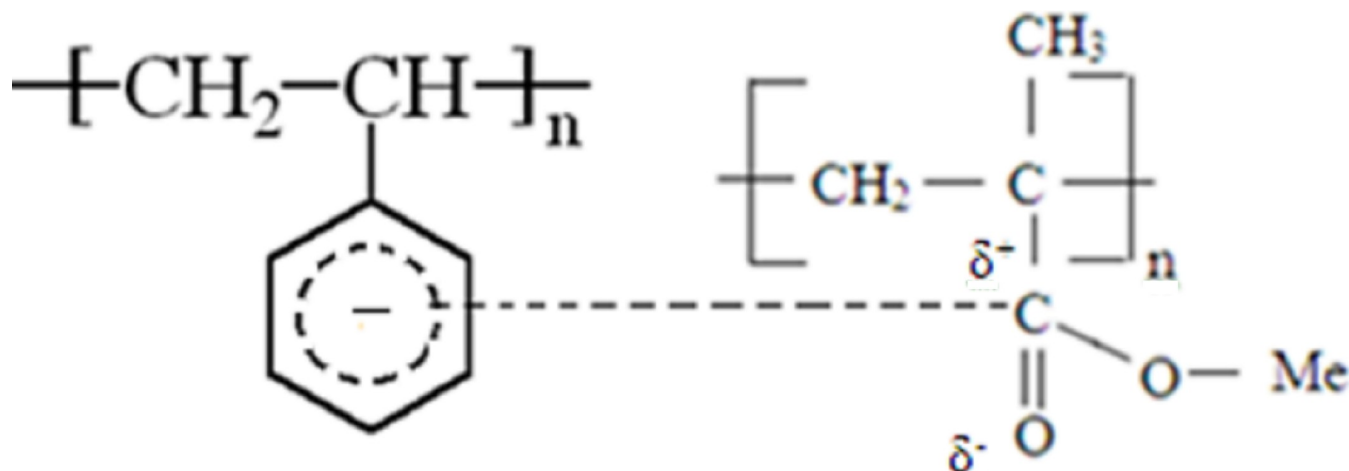


Fig 1. The mechanism of interaction that occurs between PS and PMMA.

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sustained drug delivery applications. The present study proposes about the blend formation between PS and PMMA based on the dipole interaction between aromatic groups of PS with that of the carbonyl group in PMMA. The schematic representation of the same is given in the Fig 1.

Based on the literature survey and to the best of our knowledge we are the first to report about the combination of PMMA/PS/Si composite material for medical application. The silica was selected as filler material due to its biocompatibility and ease availability in nature. There were quite an enormous amount of research that have been conducted with silica based polymeric materials for biomedical applications such as implants and artificial skin [18, 19]. Apart from being biocompatible, it also enhances the strength of polymeric materials and influence in creating surface that is suitable for apatite formation by interacting with negatively charged silanol and SBF solution. In the present study, PMMA/PS/Si composite membranes were prepared in different weight ratios of PMMA and PS (1:1, 1:2, 2:1) with constant weight of silica. The prepared composite membranes were tested for its bioactivity by immersing in SBF and cytotoxicity studies by MTT method.

Materials and methods

The materials that were used for the study were procured commercially from different sources. PMMA (Mol. Wt 35,000 Da) was procured from Asian acrylates, Mumbai, Polystyrene from Sigma Aldrich. Silica and Tetraethyl orthosilicate (TEOS, 99.5% pure) were purchased from Sigma Aldrich. THF, Hydrochloric acid was purchased from Merck. Different polymer system employed and their corresponding code, weight ratio is shown in Table 1, in all the case silica concentration was kept constant.

Table 1. Weight ratios of the polymers in their blends with 0.025g of silica.

System	Groups	Weight ratio of polymers
PMMA/PS	Group 1	1:1
	Group 2	1:2
	Group 3	2:1

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Preparation of composite membranes

The polymer silica composite membranes were prepared by weighing different amounts of PMMA and Polystyrene in separate beakers followed by dissolving in THF solvent. The polymer solutions were mixed together with constant stirring to form PMMA/PS blends. The silica particles as mentioned in Table 1 were added to the homogenous polymer solution and subsequently stirred, ultra-sonicated for 2 hours to get uniform dispersion of silica particles. In order to enhance the gelation process 0.25 ml of 35% HCl, 5 ml ethanol and 1ml of tetra ethoxy silane (TEOS) were added to the polymer solution. The solutions were left for three days stirring to complete the gelation process. The gel obtained was then casted onto clean-dried petri dishes and left undisturbed for three days at room temperature. After complete removal of solvent, the membranes were removed from the petri dish for the further analysis.

Fourier Transform Infra-Red Spectroscopy (FTIR)

The presence and interaction of different functional groups in polymers were studied using FTIR. The composite membranes of PMMA/PS prepared were characterized using Perkin Elmer Spectrum RXI IR spectrophotometer.

Scanning Electron Microscope (SEM)

The surface morphology and dispersion of silica fillers in the prepared composite membranes were studied using HITACHI S-3400N Scanning Electron Microscope (SEM). Prior to the analysis, the samples were dried and their surface was gold sputtered.

Water absorption

Water absorption property of the polymer composite membranes was carried out as per our previous studies[20]. The study was carried out by immersing pre-weighed dried membranes in deionized water on a glass beaker for 24 hours. After 24 hours, wet membranes were retrieved from the beaker and excess water was blotted gently using a tissue paper. The wet samples were again weighed, and the water absorption percentage was calculated using the formula.

$$\% \text{ Water absorption} = \frac{\text{wt. of wet polymer} - \text{wt. of dry polymer}}{\text{wt. of dry polymer}} \times 100$$

Bioactivity study

The bioactivity of the membranes prepared was studied by immersing the membranes in simulated body fluid (SBF) for 15 days (with and without silica fillers) and analyzing their surface for the formation of mineral (hydroxycarbonate apatite) layer using SEM (Quanta 200 FEG scanning electron microscope). SBF also known as Kokubo's Solution was prepared according to the specification given by Kokubo et al [21]. The chemicals required to prepare SBF were dissolved in deionized water in the specified quantity while the pH was maintained at 7.25.

Drug release kinetics

The effective drug release kinetics of composite membranes prepared were tested using 5-Fluorouracil as model drug. The solution was prepared by dissolving 12 mg 5-FU in 1000ml of deionized water. The composite membranes prepared were weighed and cut into 1cm² followed by immersion in drug solution for 24 h. The increase in weight of sample immersed after 24 h was measured and compared with initial weight to determine amount of drug loaded

onto the membrane. The amount of drug released from composite was evaluated using a UV-Visible spectrophotometer (T90+UV/Vis Spectrometer, PG Instruments) at a wavelength of 293 nm. The drug release kinetics was determined from the drug absorbance values after immersion in PBS solution and comparing it with the standard values obtained in the initial composites [22].

Biocompatibility test

The composites prepared must be biocompatible such that it can be applied *in vivo*. Hence, in the present study we used MTT assay to quantify the biocompatibility of prepared composites. The cell viability was determined based on our previous studies [23]. The biocompatibility analysis was performed using RAW macrophage cell line 264.7 that were grown on Dulbecco's Modified Eagle's Medium in a 96 well plates. The cells were allowed to settle after which the samples were added and the viability ratio was analyzed based on absorbance values at 545nm. The obtained values were plotted against the standard control group and viability ratio were estimated.

In vitro inflammatory studies

We have evaluated the inflammatory response of PMMA-PS composited using RAW 264.7 macrophage cells *in vitro*. The cells were tested with composite prepared and inflammatory responses for cytokines TNF- α , IL-6 and IL-1 were checked using qPCR. Briefly, the cells were treated with the samples and allowed for 6h incubation. The first inflammatory response was analyzed by extracting the RNA and reverse transcribing the same to form cDNA. Followed by the analyzing of expression of inflammatory markers using qPCR.

Statistical analysis

All the experiments were repeated for five times and average from them was used for the study.

Results and discussion

FTIR

The FT-IR Spectra showed the shifting of functional groups towards the lower frequency from their native frequency due to blending of polymer resulting in the formation of weak hydrogen bond.

The blend containing 1:1 ratio showed characteristic absorption peaks of PMMA corresponding to asymmetric stretching of CH₃ and C = O were assigned to bands 2951 cm⁻¹ and 1736 cm⁻¹ (Fig 2). The peaks 1482 cm⁻¹ is a characteristic vibrational band for CH₂ scissoring, similarly the vibrational band at 1452 cm⁻¹ corresponding to asymmetric vibration of CH₃ stretching or due to the PMMA deformation which was further confirmed by OCH₃ deformation peaks at 1390 cm⁻¹ (Fig 2). In addition, the absorption peaks at 1600 and 698 cm⁻¹ in all the blends represents the C-C stretching and ring deformation of polystyrene (S1 Fig). The broadened peaks at 750, 1050 and 1200 were observed for blend of 1:1 which are correspond to CH₂ twisting, wagging and rocking respectively, whereas the blend of 2:1 and 1:2 appeared to be smooth curve in the same region [24]. The IR results confirm that the blend was strongly mixed and formation of polymer blends in case of equal concentration of polymer compared to other two concentrations. It was observed that the results were in par with other reports and confirmed that there was no major shift in the peaks hence the blends may be due to physical bonding rather than a chemical one [25].

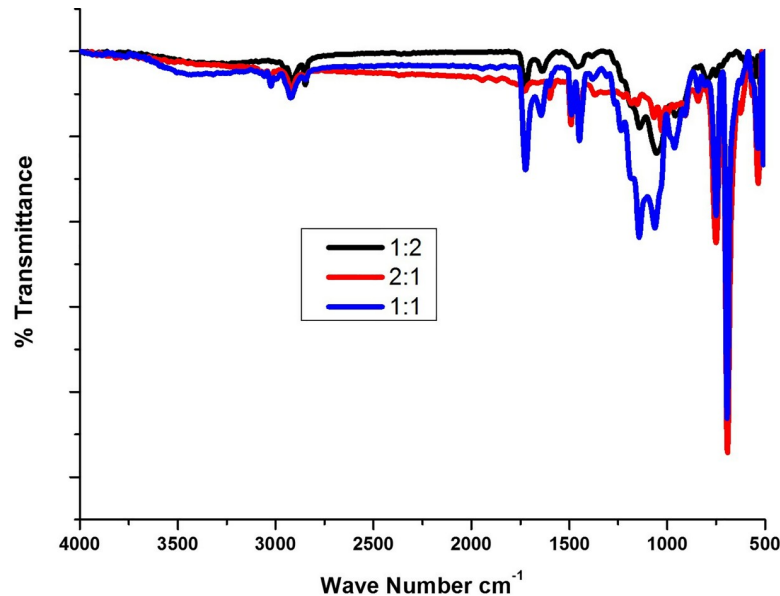


Fig 2. FTIR analysis of showing the interaction between PMMA and PS.

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Water absorption studies

The results of the water absorption studies for the three groups is shown in Fig 3. Fig 3 shows that the composite membrane of PMMA/PS 2:1 has the maximum water absorption while the membrane of 1:2 has the least water absorption (S3 Fig). This variation is attributed to the hydrophobicity of polystyrene. Hence, it was observed with increase in concentration of polystyrene decrease in water absorption capacity of the prepared composite membranes. The

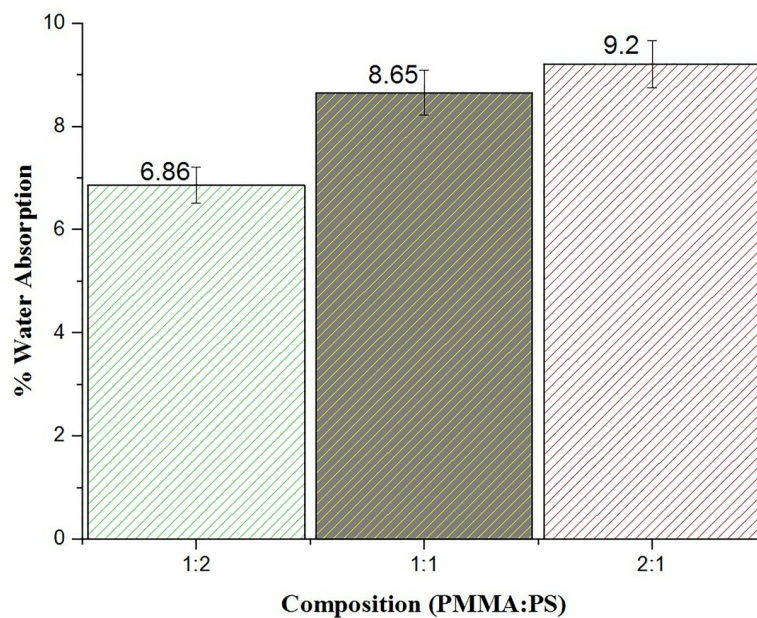


Fig 3. Percentage water absorption of the prepared composite membranes.

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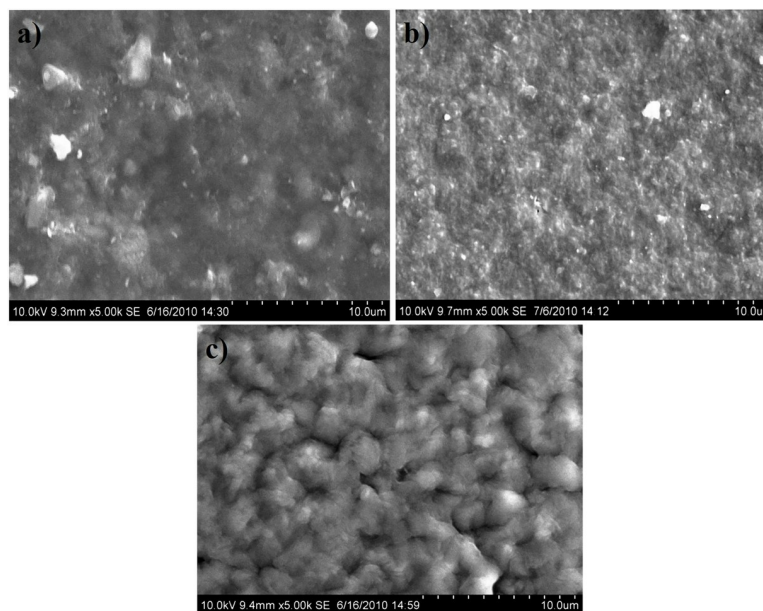


Fig 4. SEM images of a) PMMA:PS (1:2) b) PMMA:PS (1:1) c) PMMA:PS (2:1).

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swelling capacity of membranes increases with decrease in the dimensional stability, which in turn directly influence the release of drug from membrane.

Surface morphology–SEM analysis

The SEM images of the composite samples are shown in Fig 4. From the images, it was clearly seen that in all the samples, the silica fillers are evenly distributed throughout the polymer matrix.

The images showed some cracks on the surface of the composites, which may be attributed to the capillary pressure that might have occurred during the solvent evaporation. The presence of these cracks on the surface will be advantageous for drug delivery and tissue engineering applications. The presence of these pores and cracks on the surface will help in the ingrowth of the hydroxyapatite crystals thus enabling good mechanical and chemical bonding of the bone with the polymer surface.

Tensile strength

The tensile strength of the prepared samples is shown in Fig 5. From the Fig 5, it is observed that the sample of sub group 3 having PMMA: PS ratio of 2:1 has the maximum tensile strength of 78.3 MPa. With a decrease in the concentration of PMMA in blend membrane, the tensile strength is also found to decrease gradually due to elastic nature PMMA possess(S2 Fig).

Bioactivity study

Bioactivity of the prepared composite membranes was determined by keeping the membranes immersed in Kokubo solution and observing the growth of the apatite under the scanning electron microscope. The SEM images are shown in Fig 6.

The SEM images of the three subgroups after immersion in SBF for 15 days at room temperature is shown in Fig 6 that correspond to with and without the silica fillers in composites.

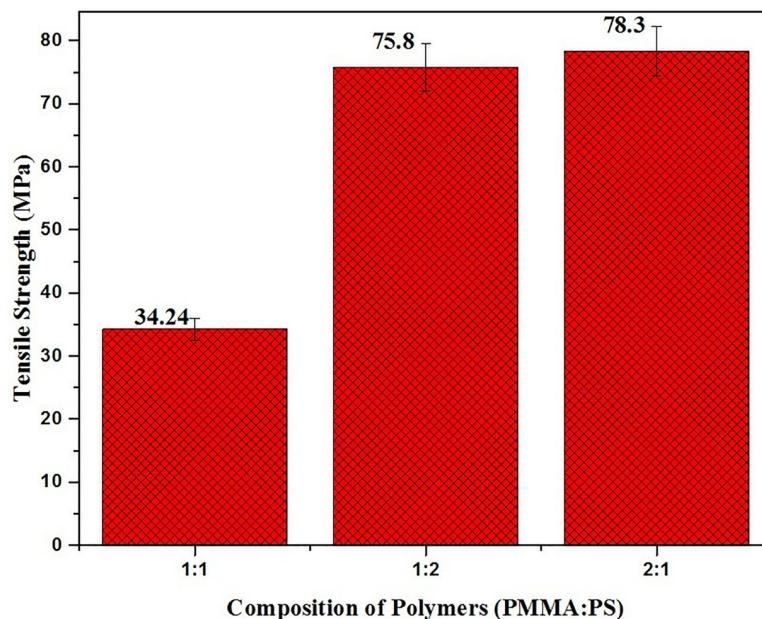


Fig 5. Tensile strength of the prepared composites.

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It was clear from Fig 6A, 6B and 6C that all the composites incorporated with Si fillers showed excellent bioactivity compared with to the ones that were not incorporated with Si. It was clear from the SEM that the Si enhance the bioactivity of membranes and it was observed that PS as well plays an important role in the bioactivity. It was observed from the Fig 6A, 6B and 6C that the composites with equal concentration of PMMA and PS were highly bioactive and the composite with more wither of PMMA or PS showed comparatively lower amount of bioactivity. The bioactivity of composites with more concentration of PS was less bioactive compared to other two composites respectively.

Drug release kinetics

The composites prepared for applying as bone cement was checked for its effective drug release kinetics as it may serve for multipurpose in enhancing the bone growth with active release of drug (Fig 7). It was observed from drug release kinetics that the drug was released in a sustained manner for a period of 180 h in PBS at pH 6.8. The initial phase or burst release was observed in all three cases the models that is in par with other research reports [26–29]. It has to be noted that the drug release in model 1 may be due to fickian diffusion and took a very long time owing to higher concentration & hydrophilic nature of PMMA that makes the drug release in a much-sustained fashion. This was not the case with model 2 & 3, which exhibited a quick release of drug compared to model 1, and this can be attributed to the complete degradation between the blended membranes since it was not a chemical bonding between the two polymers. The release kinetics were in par with FTIR results and confirmed the presence of physical interaction between polymers that might have resulted in quick release of drug from composites.

The release kinetics were fitted onto peppas model to further be confirmative about the type of release that might have occurred from composite models [29]. The model 1 had a value of $n \geq 5$ when fitted onto peppas model attributing to the fickian diffusion, the other two composite did not fit into any other models, which is out of the scope of our study.

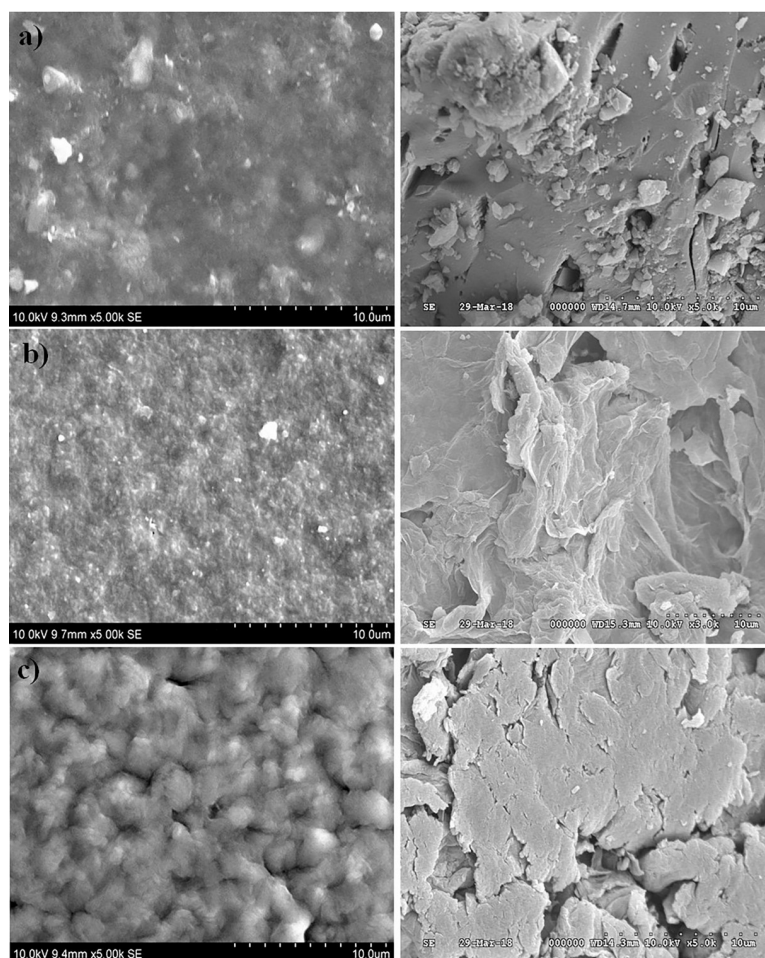


Fig 6. Shows the apatite growth on the surface of the subgroups 1, 2, 3 of PMMA:PS with silica fillers.

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***In vitro* cytotoxicity by MTT method**

The cytotoxicity studies performed showed interesting results. Fig 8 shows the RAW macrophage 264.7 cells after treating with composite membranes for 48 h. It can be observed that the cells treated with equal concentration of PMMA: PS showed confluent cells compared with the other two concentrations. It can be visually observed that amount of viable cells very lower in case of higher concentration of either PMMA or PS. Fig 8B shows high density of macrophage cells indicating its compatibility for cell proliferation.

The results were in par when studied with MTT assay where the composites with equal concentration of polymers showed an enhanced viability compared to the other two models. The percentage viability of the cells was calculated after noting the OD values of the control. The results are displayed in Fig 9 in which a graph is plotted by taking the cell count of the control group as 100 percentage. The viability percentage was in the range of 93.5 ± 3 in case of equal concentration of polymer whereas it was lower on the other two cases. Among the three groups, the sub group 2 has the best biocompatibility among other subgroups of PMMA: PS.

***In vitro* inflammatory response**

The importance of these composites when applied *in vivo* has to be taken into consideration since most of them cause inflammation when applied *in vivo* or during the application as

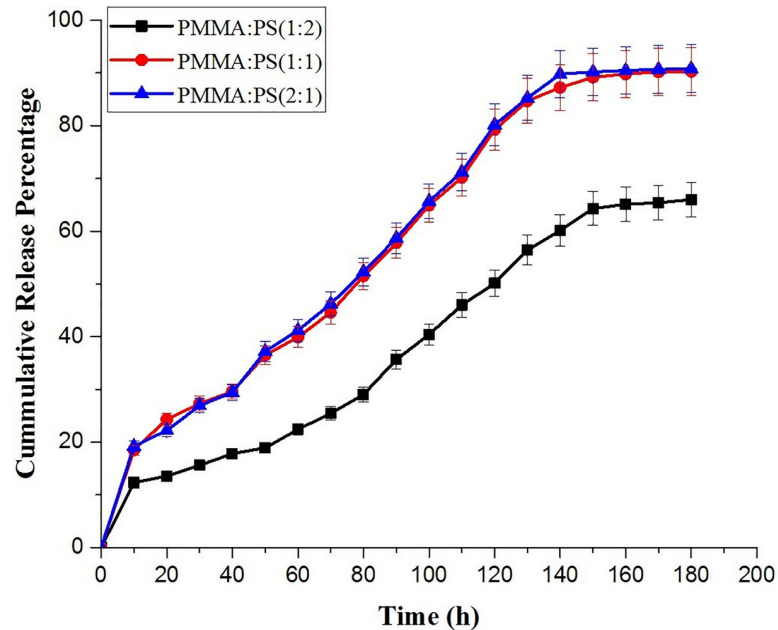


Fig 7. Shows the drug release kinetics of the prepared composite materials with varying concentration of PMMA:PS.

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prosthesis. Hence, in the present study, we evaluated inflammatory response of these composites on RAW macrophage cells and its effect was analyzed with inflammatory specific genes like $\text{TNF-}\alpha$, IL-6 and IL-1 (Fig 10). It was observed from the results that most of composites were not causing a major inflammation in all the three cases and in particular the expression was least in case of equal concentration of PMMA and PS. It was also observed that the expression of IL-6 marker gene was at a higher end in case of higher concentration of PMMA. The

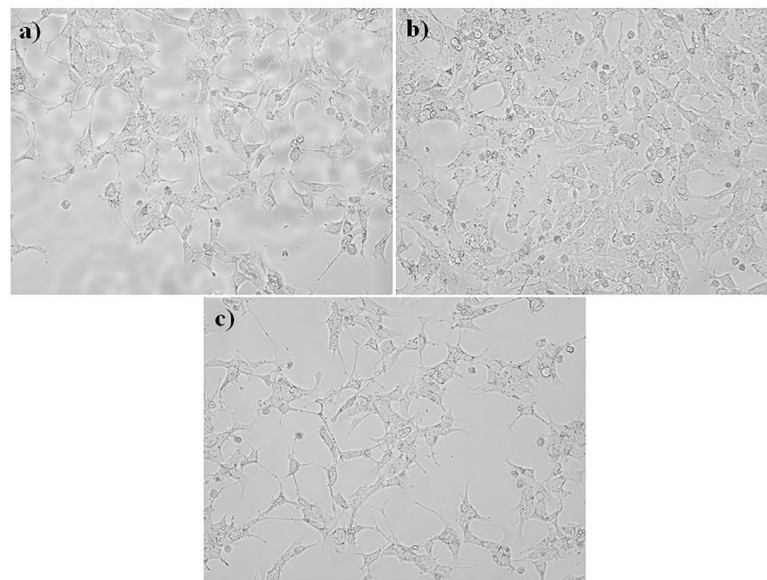


Fig 8. Microscopic images of cytotoxicity study. a) Model 1, b) Model 2, c) Model 3.

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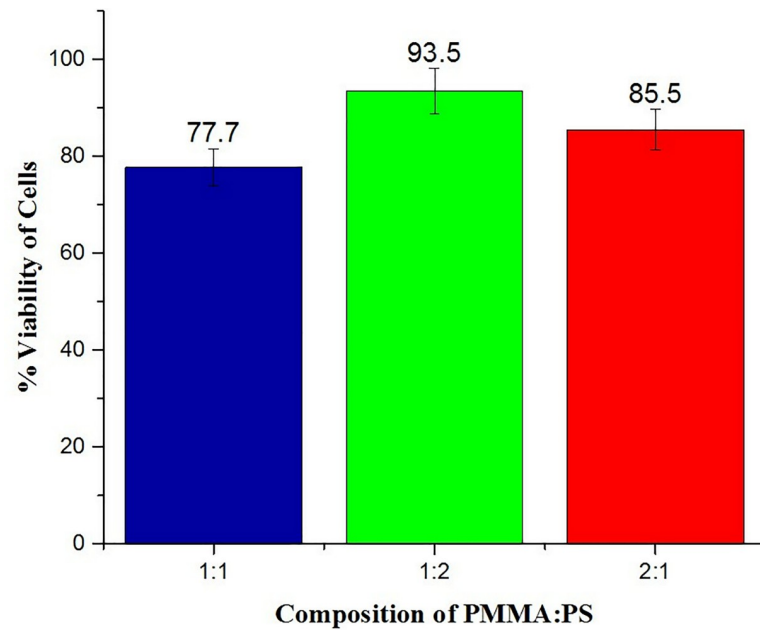


Fig 9. Graphical representation of the cell viability of the three groups.

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results from qPCR confirmed that the concentration has a direct role in causing inflammation particularly in dental applications that is in par with the one reported by Spasojevic et al [14] as they. It can be observed that these onflammatory expression was at lower end with the addition of PS only at a particular concentrations. When the concentration of PS was increased the inflammatory resposne as well got increased slightly compared to the equal concentrations. Hence, it is evident that only at equal concentration of PMMA and PS the composites very

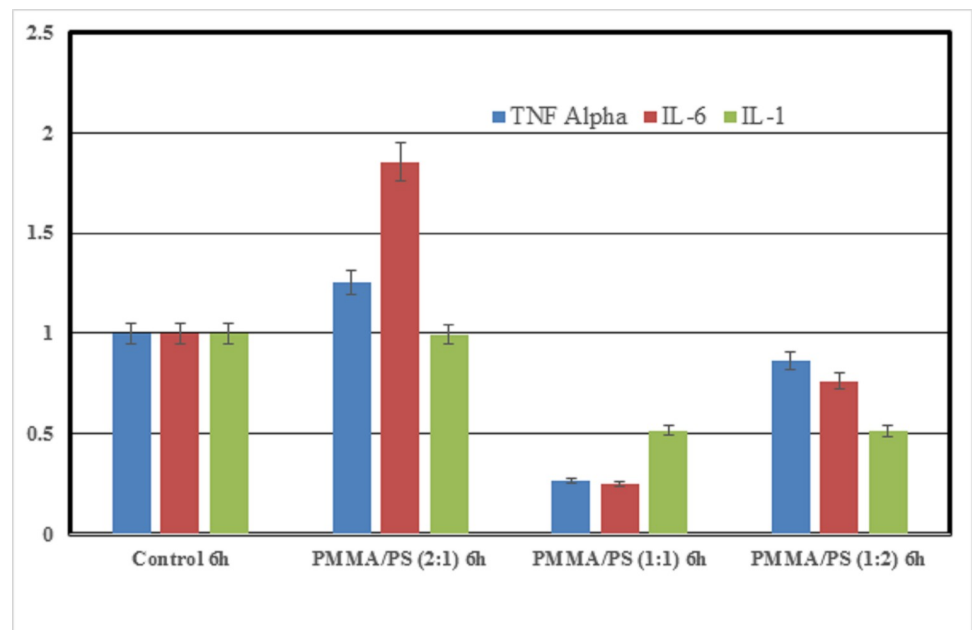


Fig 10. Inflammatory response observed on RAW macrophage cells-in vitro.

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good properties. The increase in concentration of PMMA or PS was not good property as that of the equal concentration. Therefore, the composites with equal concentration of PMMA-PS can be suitable for bio medical applications.

Conclusion

Polymer silica composites of PMMA/PS were successfully fabricated by sol-gel technique. SEM and FTIR characterized the blend membranes and confirmed the presence of physical bonding between them. The SEM images showed that the surface homogeneity of the membranes was dependent on the concentration and blending between the two constituent polymers. The blend formation between the polymers in the three groups was confirmed by FTIR. The bioactivity of membranes were analyzed by immersing the samples in SBF and examining the surface for the formation of apatite layer under the scanning electron microscope (SEM). Among the three groups, model 1 containing equal concentration of PMMA/PS blend composite showed the high bioactivity while the group two showed the most dense apatite formation of Ca-P crystals on its surface. The cytotoxicity studies were performed to evaluate the biocompatibility of the fabricated composite membranes. The *in vitro* study carried out by MTT method showed the composite with equal concentration of group II and III samples exhibited excellent cell viability while the group I, the sample of the subgroup 3 namely PMMA:PS in the ratio of 2:1 exhibited favorable cytotoxicity value than the other two subgroups. From this study, it can be concluded that the sol-gel technique is versatile method to fabricate the polymer composites having immense biomedical application. Among the polymers investigated, model 1 samples were less suited for load bearing orthopedic applications but more suited for sustained drug delivery and tissue engineering application. Model 1 showed very good tensile properties whereas other two models showed optimum biocompatibility and moderate strength that shall be suitable orthopedic application.

Supporting information

S1 Fig. The data set of FTIR.
(OPJ)

S2 Fig. The data set of tensile strength.
(OPJ)

S3 Fig. The data set of water absorption.
(OPJ)

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Writing – review & editing: S. Shanmugasundar, V. Prabhu, N. Balasubramanian.

References

1. Qazi TH, Mooney DJ, Pumberger M, et al. (2015) Biomaterials based strategies for skeletal muscle tissue engineering: Existing technologies and future trends. *Biomaterials* 53:502–521. <https://doi.org/10.1016/j.biomaterials.2015.02.110> PMID: 25890747
2. Park O, Yu G, Jung H, Mok H (2017) Recent studies on micro- / nano-sized biomaterials for cancer immunotherapy. *J Pharm Investig* 47:11–18. <https://doi.org/10.1007/s40005-016-0288-2>
3. Balint R, Cassidy NJ, Cartmell SH (2014) Acta Biomaterialia Conductive polymers: Towards a smart biomaterial for tissue engineering. *Acta Biomater* 10:2341–2353. <https://doi.org/10.1016/j.actbio.2014.02.015> PMID: 24556448
4. Miao S, Wang P, Su Z, Zhang S (2014) Acta Biomaterialia Vegetable-oil-based polymers as future polymeric biomaterials q. *Acta Biomater* 10:1692–1704. <https://doi.org/10.1016/j.actbio.2013.08.040> PMID: 24012607
5. Gandhi A, Paul A, Sen SO, Sen KK (2015) ScienceDirect Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications. *Asian J Pharm Sci* 10:99–107. <https://doi.org/10.1016/j.ajps.2014.08.010>
6. Park J, Lakes RS (2007) *Biomaterials*, 3rd ed. Springer
7. Ahamed MIN, Sankar S, Kashif PM, et al. (2015) International Journal of Biological Macromolecules Evaluation of biomaterial containing regenerated cellulose and chitosan incorporated with silver nanoparticles. *Int J Biol Macromol* 72:680–686. <https://doi.org/10.1016/j.ijbiomac.2014.08.055> PMID: 25224288
8. Logithkumar R, Keshavnarayan A, Dhivya S, et al. (2016) A review of chitosan and its derivatives in bone tissue engineering. *Carbohydr Polym* 151:172–188. <https://doi.org/10.1016/j.carbpol.2016.05.049> PMID: 27474556
9. Aravind K, Sangeetha D (2014) Characterization and In Vitro Studies of Sulfonated Polyether Ether Ketone/Polyether Sulfone/Nano Hydroxyapatite Composite. *Int J Polym Mater Polym Biomater* 64:220–227. <https://doi.org/10.1080/00914037.2014.936594>
10. Boccaccini AR, X.Ma P (2014) *Tissue Engineering Using Ceramics and Polymers*, 2nd ed. Woodhead Publishing Series, United Kingdom
11. Dorney J (2013) Polystyrene: A Potential Standard for Developing In Vitro Cellular Tracking Methods for Nanotoxicology. <https://doi.org/10.21427/D75C76>
12. Aravind Bhat K, Prakash PL, Niranjanaa M, Lakshmi Bai A (2013) Fabrication of Polymethyl Methacrylate / Polysulfone / Nanoceramic Composites for Orthopedic Applications. *J Appl Polym Sci* 2764–2775. <https://doi.org/10.1002/app.37581>
13. Kansu G., Kalyoncuoğlu T., Uyar P., and Uzun E., “Cell death induced by eluates from hypoallergenic denture base acrylic resins in NIH-3T3 fibroblast cells,” *Journal of Dental Sciences*, vol. 9, pp. 381–387, 2014.
14. Spasojevic Pavle, Zrilic Milorad, Panic Vesna, Stamenkovic Dragoslav, Seslija Sanja, and Velickovic Sava, “The Mechanical Properties of a Poly(methyl methacrylate) Denture Base Material Modified with Dimethyl Itaconate and Di-n-butyl Itaconate,” *International Journal of Polymer Science*, vol. 2015, Article ID 561012, 9 pages, 2015. <https://doi.org/10.1155/2015/561012>.
15. Barbosa-stancioli EF, Pereira MM, Orefice RL, et al. (2008) Sol–gel derived composite from bioactive glass–polyvinyl alcohol. *J Mater Sci* 43:494–502. <https://doi.org/10.1007/s10853-007-1875-4>

16. Schmidt H, Jonschker G, Goedicke S, Mennig M (2000) The Sol-Gel Process as a Basic Technology for Nanoparticle-Dispersed Inorganic-Organic Composites. *J Sol-gel Sci Technol* 19:39–51.
17. Jing Y, Wei G, Huang X, et al. (2008) Sol-gel derived mesoporous bioactive glass fibers as tissue-engineering scaffolds. *J sol-gel Technol* 20:115–119. <https://doi.org/10.1007/s10971-007-1668-x>
18. Aravind K, Sundar SS, Sangeetha D (2014) In Vivo Studies of Sulphonated Polyether Ether Ketone Based Composite Bone Graft Materials. *Trends Biomater artificial organs* 28:52–57.
19. Dziadek M, Stodolak-zych E, Cholewa-kowalska K (2017) Biodegradable ceramic-polymer composites for biomedical applications: A review. *Mater Sci Eng C* 71:1175–1191. <https://doi.org/10.1016/j.msec.2016.10.014> PMID: 27987674
20. Prabhu N. V. Sangeetha D (2015) Effect of zeolite on SPEEK /zeolite hybrid membrane as electrolyte for microbial fuel cell applications. *RSC Adv* 5:84004–84013. <https://doi.org/10.1039/C5RA14701H>
21. Kokubo T., Kushitani H., Ebisawa Y., Kitsugi T., Kotani S., Oura K. and Yamamuro T., (1989). Apatite formation on bioactive ceramics in body environment. *Bio ceramics*, 1, pp.157–162.
22. Kannan N., Shanmuga Sundar., Balaji S., Arul Amuthan L., Anil Kumar N V., Balasubramanian N., (2018) Physiochemical characterization and cytotoxicity evaluation of mercury-based formulation for the development of anticancer therapeutics. *PLoS One*, 13 (04). 01–13. ISSN 1932-6203
23. Kanniappan K and Latha S, (2011) Certain Investigations on the Formulation and Characterization of Polystyrene / Poly(methyl methacrylate) Blends. *Int.J. ChemTech Res.* 2011, 3(2). 708–715.
24. Radhakrishnan Nair M.N., Thomas G.V. and Gopinathan Nair M.R., Miscibility Studies of PVC / ELNR Blends, *Polymer Bulletin*, 2001, 46, 191–196.
25. Streubel J, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. *Int J Pharm*, 2002; 241:279–92. PMID: 12100855
26. Streubel J, Siepmann J, Bodmeier R. Multiple unit gastroretentive drug delivery systems: A new preparation method for low density microparticles. *J Microencapsulation*, 2003; 20:329–47. <https://doi.org/10.1080/0265204021000058384> PMID: 12881114
27. Yuksel N, Baykara M, Shirinzade H, Suzen S. Investigation of triacetin effect on indomethacin release from poly(methyl methacrylate) microspheres: Evaluation of interactions using FT-IR and NMR spectroscopies. *Int J Pharm*, 2011; 404:102–9. <https://doi.org/10.1016/j.ijpharm.2010.11.011> PMID: 21093553
28. Dhana lekshmi UM, Poovi G, Kishore N, Reddy PN. In vitro characterization and in vivo toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. *Int J Pharm*, 2010; 396:194–203. <https://doi.org/10.1016/j.ijpharm.2010.06.023> PMID: 20600729
29. Sundar S. S.; Sangeetha D. Fabrication and evaluation of electrospun collagen/poly (N-isopropyl acrylamide)/chitosan mat as blood-contacting biomaterials for drug delivery. *J Mater Sci-Mater M.* 2012, 23, 1421–1430.