A Novel Mesoporous Ca-Si-Ti-Sr Blended Pmma Hybrid Scaffolds Adopted by Thermal Induced Phase Separation Technique (Tips): Analysis of its Chemical and Biological Properties

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**Abstract:** The blend of calcium (Ca), silicon (Si), titanium (Ti), and strontium (Sr) offers the potential for enhanced mechanical performance and improved biological response. PMMA- as a base polymer is added to increase the strength of the scaffold and has well-established biocompatibility, ease of fabrication, and desirable mechanical properties.Blending PMMA with the Ca-Si-Ti-Sr components,gives synergistic effects of these elements, which are known for their osteogenic and angiogenic potential.Thermal-induced phase separation (TIPS) fabrication technique creates a well-defined porous structures with interconnected pores.The mesoporous nature of the scaffold provides a favorable environment for cell adhesion, proliferation, and differentiation, promoting tissue regeneration processes.Calcium nitrate (Ca(NO3)2), tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TMOS), titanium isopropoxide (TTIP), and strontium nitrate (Sr(NO3)2) precursors of Ca,Si,Ti,Sr were taken.Bioglass solution was prepared using Sol gel method15% PMMA is dissolved in AcetoneScaffolds are prepared by TIPSEqual volumes of the transparent Bioglass solution and the polymer mixtures were added to and were stirred overnight with heat treatmentThe heat treatment was essential since the dissolvability of PMMA in these solvent mixtures at room temperature is very low.The stirred solution is kept in the oven to evaporate the solvent at 65 degrees and the scaffold is obtainedSEM analysis shows that the bioglass has an irregular shape and is crystalline in nature and PMMA has a plane surface and the scaffold has bioglass incorporated in the surface of PMMA.The surface roughness of Scaffold is measured by AFM and the scaffold has surface area of 17.982 nm and PMMA has a surface area of 4.9307nm. EDAX analysis confirmed the presence of O,Ca, Sr, C,Ti and Si elements. Chemical bindings , functional groups and purity of the sample are checked using FTIR analysis.Different peaks observed in the bioglass are similar to peaks seen in PMMA/bioglass. The scaffold has extra peaks representing carbon linkages in PMMA. Biocompatibility of the scaffold is checked by Hemocompatibility (the haemolysis is around 2.25 percent), zebrafish embryonic toxicology (mortality rate is 1.04 percent at 96 hours), Confocal analysis( the contact angle of scaffold is 61 degrees and that of PMMA is 70 degrees).SEM analysis confirms the proper surface morphology of scaffold with desired mechanical properties is formed. AFM confirms the surface area and surface roughness of scaffold is more than PMMA. EDAX confirms the chemical elemental composition of the synthesised scaffold is correct. FTIR confirms there is no contamination and the chemical binding of bioglass and PMMA.The sample has the desired mechanical properties. The Hemocompatibility, confocal analysis and zebrafish embryonic toxicology study shows the scaffold is biocompatible and can naturally bind to cells.[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/u4vtXV/8xWv7+U86ng+8WV9j)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/u4vtXV/yPnK+1wV0+BUrq)[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/u4vtXV/l3zEk+swBJ7+iTozs)[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/u4vtXV/Gd8ur+0Htbl+l3GRL)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/u4vtXV/wctsN+k0wNX+wttLB)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/u4vtXV/wctsN+k0wNX+wttLB+YJw26)[(Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial, n.d.; Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/u4vtXV/hRgnd+YdFLZ+ikwB3)[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/u4vtXV/H1uIj+MOvSn))

**Keywords:** Hemocompatibility Assay ,Confocal Analysis, Ftir Spectrum Of Bioglass

# INTRODUCTION

Because mesoporous elements like Ca, Si, Ti, and Sr have a well-established history of fostering tissue regeneration, boosting biocompatibility, and encouraging osteogenesis, their inclusion into materials has a tremendous amount of promise [(Bacakova et al., 2018; Shanmugam et al., 2013)](https://paperpile.com/c/u4vtXV/pKmf+ghY1). These components were combined with the adaptable PMMA polymer matrix to create a scaffold with specific qualities, such as structural integrity, porosity, and bioactive potential(Saadh et al., 2024). It has been demonstrated that strontium helps mesenchymal stem cells differentiate into osteoblasts, the cells responsible for bone production [(Lee, 2010; Viishaal Srikanth Srivatsa & Manogaran, 2024)](https://paperpile.com/c/u4vtXV/QP5c+p6KB)[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/u4vtXV/8xWv7+U86ng+8WV9j)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/u4vtXV/yPnK+1wV0+BUrq)[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/u4vtXV/l3zEk+swBJ7+iTozs)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/u4vtXV/yPnK+1wV0+BUrq)[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/u4vtXV/Gd8ur+0Htbl+l3GRL).This can make the scaffold more advantageous for orthopedic and dental applications by improving its capacity to assist bone regrowth.Titanium has a very good biocompatibility reputation [(Hutmacher, 2000; Sathya et al., 2024)](https://paperpile.com/c/u4vtXV/rna9+R7s6). Due to its low risk of adverse responses, it is well tolerated by the human body and frequently utilized in medical implants, including dental implants and orthopedic prosthesis(Almatrafi et al., 2024). Due to its osteoconductive characteristics, titanium promotes bone cell development and attachment. This is essential for the scaffold's fusion with the surrounding bone tissue [(Swain & Jawaid, 2019; Thiripelu et al., 2024)](https://paperpile.com/c/u4vtXV/3aFr+6GxJ). The scaffold's porosity can be precisely controlled using TIPS. Researchers can customize the pore size and distribution to meet certain tissue engineering requirements by altering variables like freezing temperature and solvent evaporation rate. TIPS creates interconnecting pores in the scaffold that resemble the extracellular matrix found naturally in cells [(Ahmed, 2018)](https://paperpile.com/c/u4vtXV/HJ0p)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/u4vtXV/wctsN+k0wNX+wttLB)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/u4vtXV/wctsN+k0wNX+wttLB+YJw26)[(Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial, n.d.; Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/u4vtXV/hRgnd+YdFLZ+ikwB3)[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/u4vtXV/H1uIj+MOvSn))The promotion of cell infiltration, nutrition diffusion, and waste removal—all essential for tissue regeneration—is made possible by this feature [(Inamuddin & Altalhi, 2022; aramasivam et al., 2023)](https://paperpile.com/c/u4vtXV/MmnL+Eqdo). TIPS is compatible with a variety of biocompatible polymers and solvents, allowing for the creation of scaffolds suitable for different biological applications. TIPS is a relatively mild process, which helps preserve the bioactivity of any incorporated bioactive molecules, growth factors, or drugs within the scaffold. TIPS can result in scaffolds with minimal need for post-processing steps, saving time and resources in scaffold preparation [(Thakur et al., 2017)](https://paperpile.com/c/u4vtXV/P7qO).

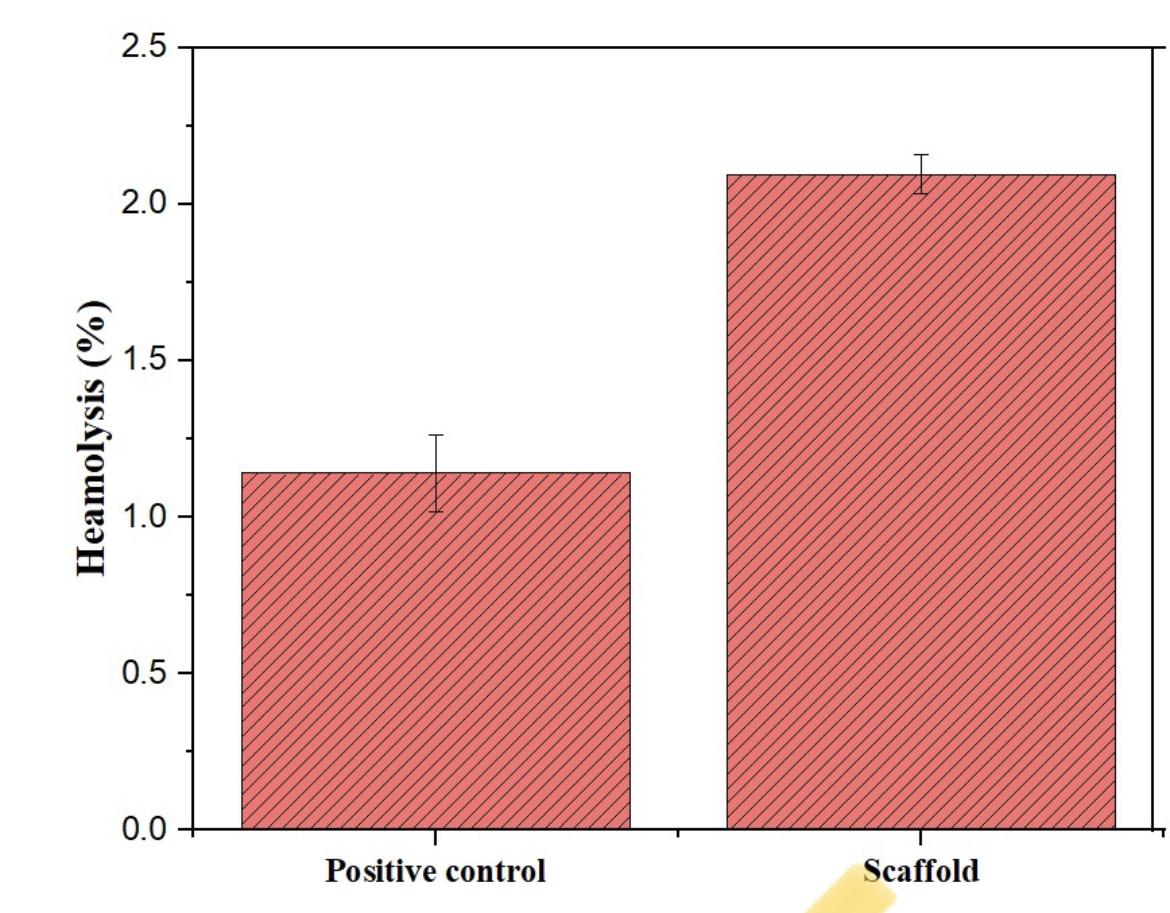
# MATERIALS AND METHODS

* Calcium nitrate (Ca(NO3)2), tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TMOS), titanium isopropoxide (TTIP), and strontium nitrate (Sr(NO3)2) precursors of Ca,Si,Ti,Sr were taken.
* Bioglass solution was prepared using Sol gel method
* 15% PMMA is dissolved in Acetone
* Scaffolds are prepared by TIPS
* Equal volumes of the transparent Bioglass solution and the polymer mixtures were added to and were stirred overnight with heat treatment
* The heat treatment was essential since the dissolvability of PMMA in these solvent mixtures at room temperature is very low.
* The stirred solution is kept in the oven to evaporate the solvent at 65 degrees and the scaffold is obtained

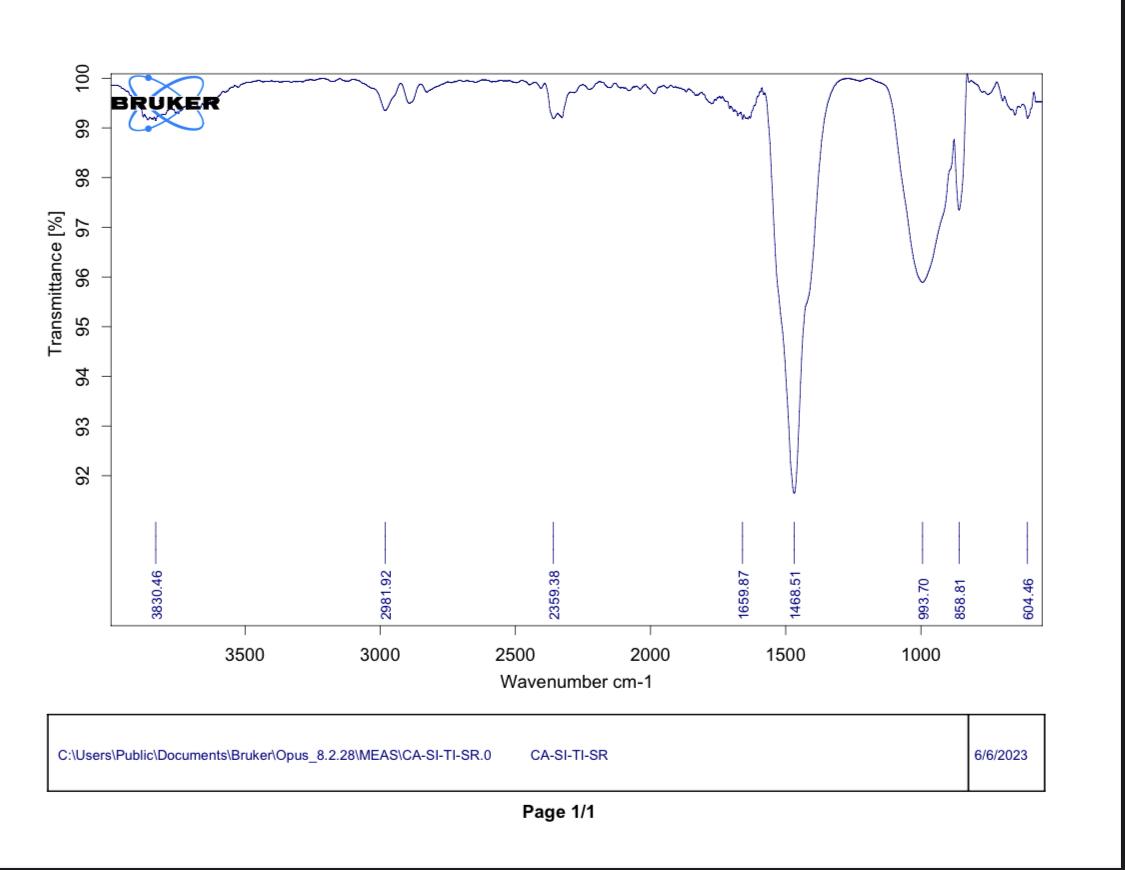
# RESULTS

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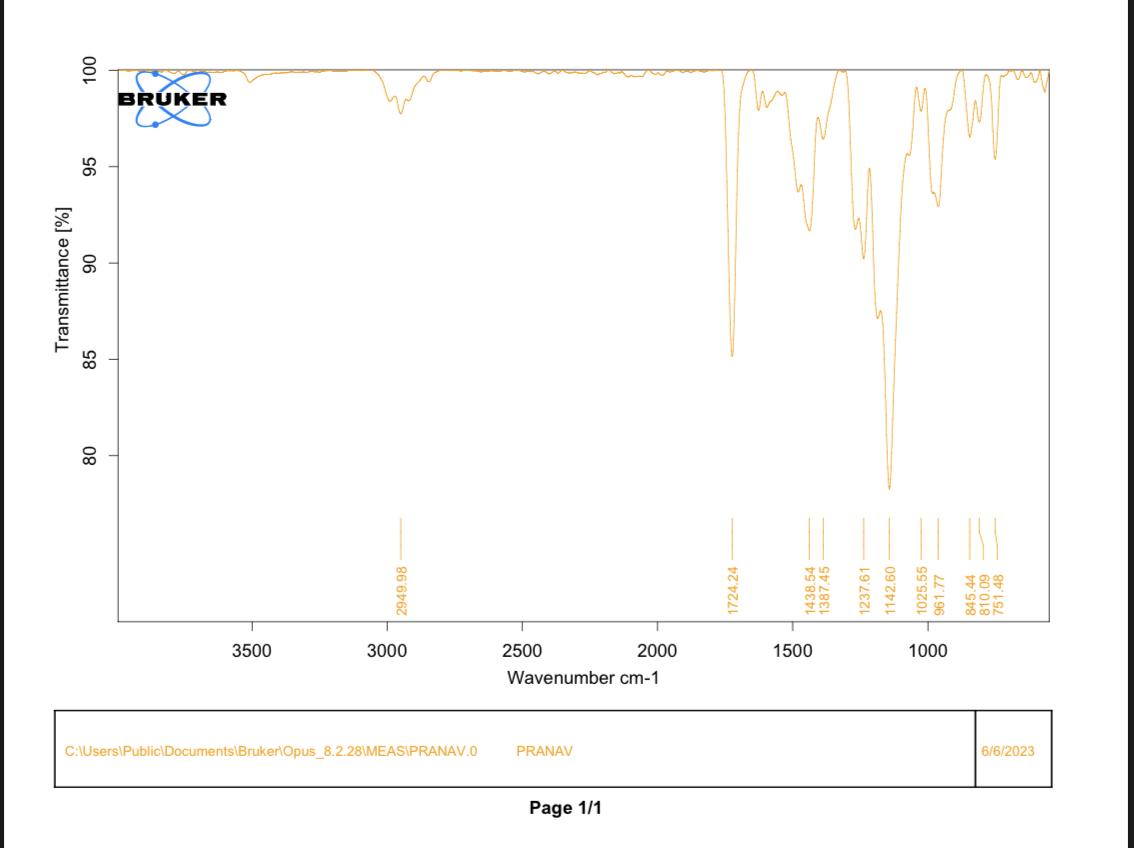
**FIGURE 1** : CONFOCAL ANALYSIS

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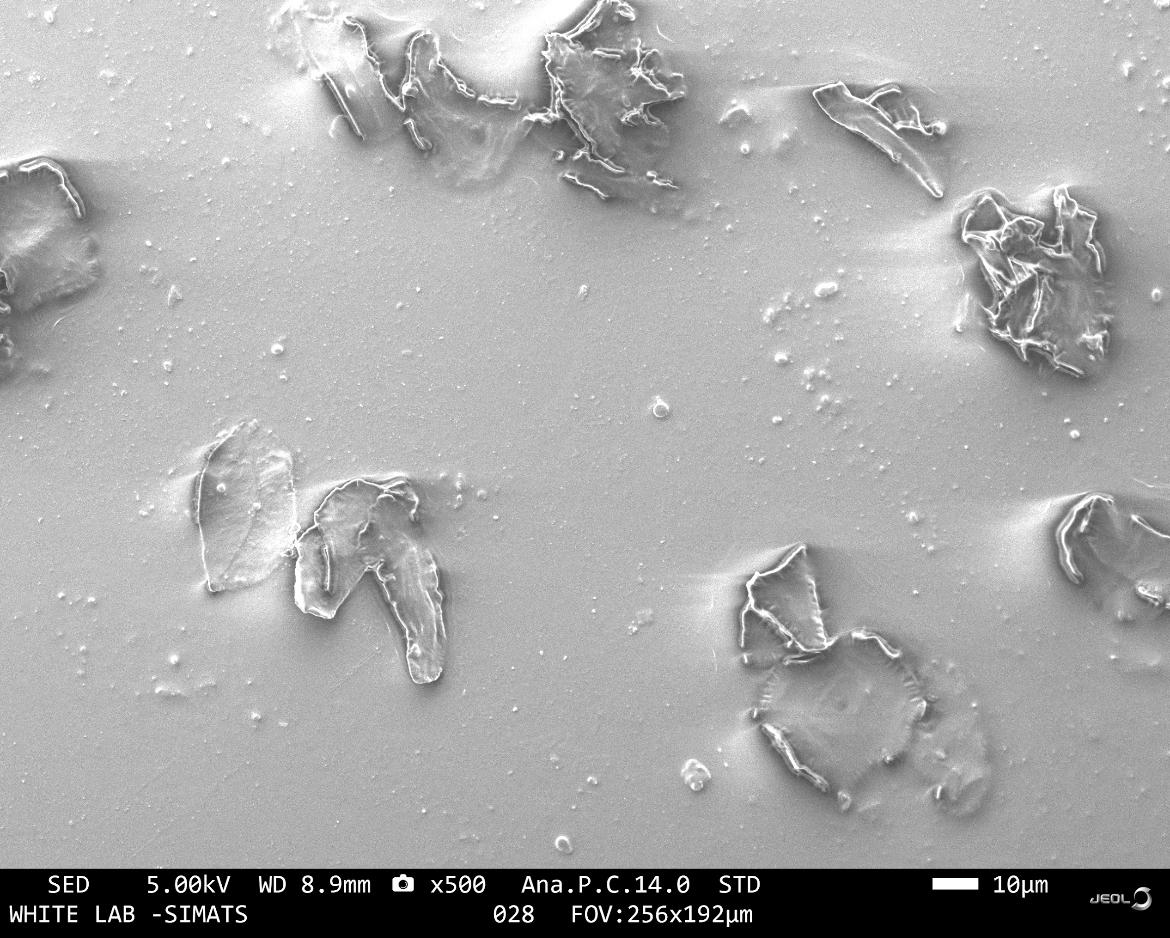
**FIGURE 2 :** HEMOCOMPATIBILITY ASSAY

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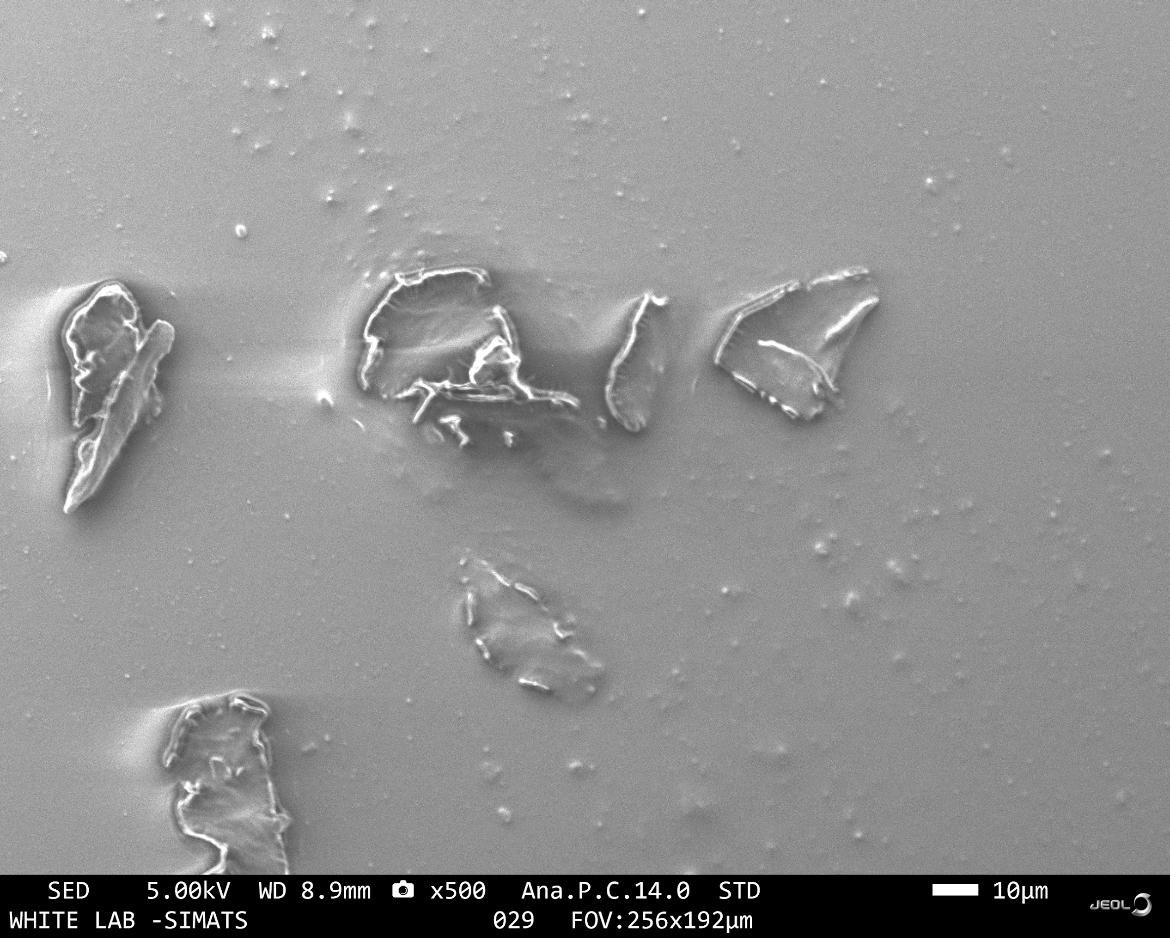
**FIGURE 3:** FTIR SPECTRUM OF BIOGLASS

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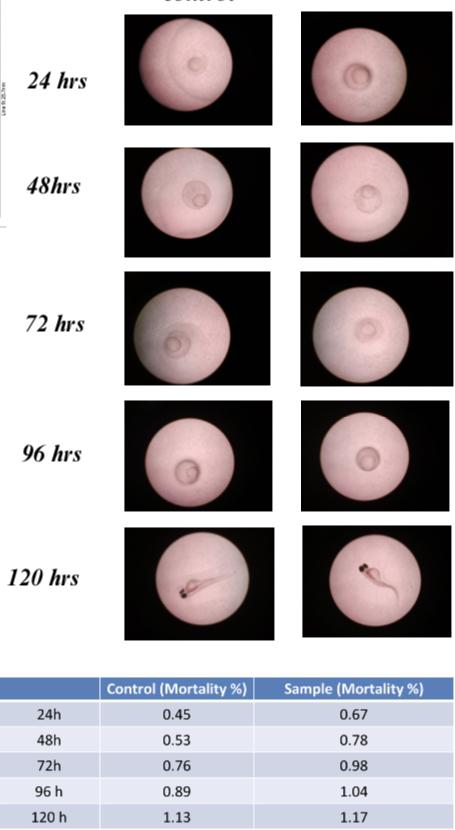
**FIGURE 4 :** FTIR SPECTRUM OF PMMA/BIOGLASS

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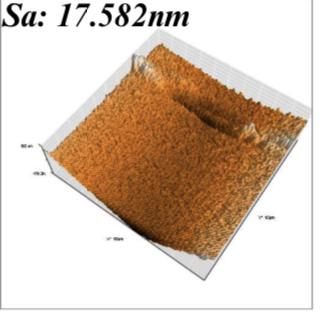
**FIGURE 5:** SURFACE MORPHOLOGY OF SCAFFOLD

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**FIGURE 6:** SURFACE MORPHOLOGY OF PMMA

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**FIGURE 7 :** ZEBRAFISH STUDY

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**FIGURE 8 :** AFM SURFACE ROUGHNESS

# DISCUSSION

The scaffold features bioglass embedded in the surface of PMMA, according to SEM research, which also reveals that the bioglass has an irregular shape and is crystalline in nature.AFM confirms the scaffold's surface roughness, and the scaffold has a surface area of 17.982 nm compared to PMMA's 4.9307 nm [(Arivarasan et al., 2021; Thakur et al., 2017)](https://paperpile.com/c/u4vtXV/P7qO+8F76). EDAX analysis provides the elemental composition of the various scaffolding elements. Using FTIR analysis, chemical ties, functional groups, and sample purity are examined.Peaks seen in PMMA/bioglass are similar to different peaks seen in the bioglass. Additional peaks on the scaffold signify the carbon links in PMMA. Confocal analysis (the contact angle of the scaffold is 61 degrees and that of PMMA is 70 degrees) and zebrafish embryonic toxicology (mortality rate is 1.04 percent at 96 hours) are used to evaluate the scaffold's biocompatibility [(Cheng, 2008)](https://paperpile.com/c/u4vtXV/CYhb).Doped ZnO and MgO into the 45S5 BG scaffolds to show enhanced compressive strength and fracture toughness. Because XLS nanosheet may serve as the filler and physical crosslinker to the polymer, the 2D Nano Silicate (Laponite®, XLS), a magnesium silicate (Na+0.7[(Si8Mg5.5Li0.3)O20(OH)4]0.7), has been found to significantly improve the mechanical properties of polymeric matrix [(McKenna et al., 2019)](https://paperpile.com/c/u4vtXV/48Zo).TIPS created PLA foams by offering various experimental conditions for polymer crystallization during liquid-liquid phase separation and the gelation process, followed by solvent exchange and vacuum drying[(Netti, 2014)](https://paperpile.com/c/u4vtXV/JpI5). Different ratios of the THF/water (solvent/nonsolvent) system were used, ranging from 84/16 to 90/10. By lowering the PLA solutions (6–10 wt%) to three different temperatures—24, 4 and 20 °C—phase separation was generated [(Netti, 2014; Sheikh, 2021)](https://paperpile.com/c/u4vtXV/JpI5+Puto). To create the porous, stiff, and highly crystalline micro- and nano-structures, the resulting PLA gels were vacuum-dried after being treated to solvent exchange (with ethanol). By adjusting the TIPS process parameters (i.e., polymer concentration, THF/water ratio, and quenching temperature), the porosity (between 85.1 and 92.8%), pore size (25-400 m), morphology, and mechanical properties of these rigid PLA foams were all adjusted [(Netti, 2014; Sanjeevi, 2019; Sheikh, 2021)](https://paperpile.com/c/u4vtXV/JpI5+Puto+z4Bj).

# CONCLUSION

SEM analysis confirms the proper surface morphology of scaffold with desired mechanical properties is formed. AFM confirms the surface area and surface roughness of scaffold is more than PMMA. EDAX confirms the chemical elemental composition of the synthesised scaffold is correct. FTIR confirms there is no contamination and the chemical binding of bioglass and PMMA.The sample has the desired mechanical properties. The Hemocompatibility,confocal analysis and zebrafish embryonic toxicology study shows the scaffold is biocompatible and can naturally bind to cells.

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