श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेंद्रम, तिरुवनन्तपुरम - 695 011, केरल, भारत
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PROJECT COMPLETION REPORT

1. **Project Number** : **8172**

2. **Title of the Project** : **Development of bioactive bone cement based on novel inorganic-organic hybrid resin**

3. **Funding Agency Name** : KSCSTE

4. **Project Reference Number provided by the Funding Agency**:
009/SRSHS/2014/CSTE dated 18/06/2016

5. **Principal Investigator (Name & Address) : Dr.Lizymol P.P. ,Scientist, DEP,DBST,BMTW,SCTIMST**

6. **Co-Investigators (Name & Address):**

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2. . Dr.V.Kalliyanakrishnan Retd.Scientist G (Senior grade)
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7. **Implementing Institution** : SCTIMST

8. **Collaborating Institutions** : VTM NSS College, Dhanuvachapuram

9. **Date of Commencement** : 30.12.2016

10. **Duration** : 3 YEARS AND 3 MONTHS (extension)

11. **Date of Completion** : 31/03 2020

12. **Objectives as approved :** To develop a MMA and BisGMA free bioactive bone cement with low polymerization shrinkage, high monomer conversion, radiopacity and enhanced

remineralization without the generation of exotherm. The proposed bone cement based on inorganic-organic hybrid resin containing shell nacre /calcium/strontium/zirconium with multifunctionality is expected to be non cytotoxic and have the ability to form strong bond formation with the implant and bone there by reducing the chance of clinical failure

13. Deviation made from original objectives if any, while implementing the project and reasons thereof : NIL

14. Field/Experimental work giving full details of summary of methods adopted, data collected supported by necessary tables, charts, diagrams and photographs :

Synthesis of Poly (methyl methacrylate-Poly (styrene) [PMMA –PS] copolymer

Reagents used

Disodium hydrogen phosphate (Merck), sodium dihydrogen phosphate, polyvinyl alcohol (Aldrich), Methyl methacrylate (Aldrich), Styrene (Aldrich)

Procedure

300 ml of distilled water ,2g polyvinyl alcohol (average mol. weight 89,000-98,000 Aldrich),1.706g disodium hydrogen phosphate and 0.10g sodium dihydrogen phosphate were taken in a three necked round bottom flask fitted with a thermowell,condenser and an inert gas inlet. The flask was provided with a thermo well, condenser, glass stirrer and an inert gas inlet, nitrogen was purged through the solution and the solution was warmed to 40°C.When the solution became clear, 100ml of washed methyl methacrylate (MMA) monomer containing 1.5g benzoyl peroxide dissolved in it and 6.13 ml styrene (6%) were added into the solution with constant stirring .Stirring was maintained at 800-1200 rpm at 70°C for 3 hours. At the end of 3 hours, temperature reached to 76°C and kept at 76°C for 20 minutes. Heating was stopped and the mixture was stirred for 1 hour and transferred to distilled water in a beaker, filtered, washed with hot water to remove poly vinyl alcohol and dried at 40° C in an air oven for 16 hours. The polymer was powdered in a centrifugal ball mill (Retsch, Germany) for 2 hours.PMMA-PS copolymer with varying styrene content (12%, 15%, 18% and 50%) was also synthesized.

Synthesis of Poly (methyl methacrylate) polymer

Reagents used

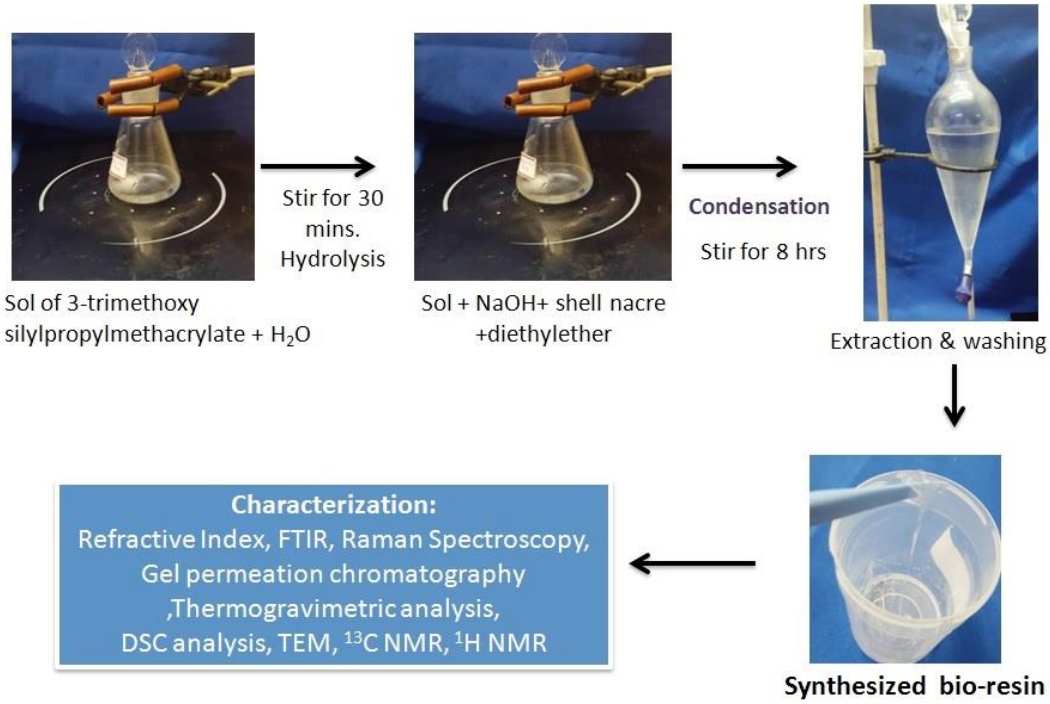
Disodium hydrogen phosphate (Merck), sodium dihydrogen phosphate, polyvinyl alcohol (Aldrich), Methyl methacrylate (Aldrich)

Procedure

480 ml of distilled water, 3g polyvinyl alcohol, 2.55g disodium hydrogen phosphate and 0.15g monosodium dihydrogen phosphate were taken in a three necked round bottom flask fitted with a thermowell, condenser and an inert gas inlet. The flask was provided with a thermo well,

condenser, glass stirrer and an inert gas inlet, nitrogen was purged through the solution and the solution was warmed to 40°C.When the solution became clear, 160ml of washed methyl methacrylate (MMA) monomer containing 1.5g benzoyl peroxide dissolved in it was added into the solution with constant stirring .Stirring was maintained at 350-400 rpm at 70°C for 1 hour. After exactly 1 hour, exotherm occurred and the temperature reached to 84°C. The reaction temperature was quenched to room temperature and stirring was continued for 1 hour .The reaction mixture was transferred to distilled water in a beaker, filtered, washed with hot water to remove poly vinyl alcohol and dried at 40° C in an air oven for 16 hours. The polymer was powdered in a centrifugal ball mill (Retsch, Germany) for 2 hours and sieved. Polymer with particle size less than 120µ was used for the preparation of bone cement.

Synthesis and characterization of calcium/ shell nacre containing siloxane methacrylate resins



Experimental setup of resin synthesis

The siloxane methacrylate resins were synthesized by the modified sol gel method using the precursor 3-TMSPM. The bare resin without any inorganic content is taken as control resin. Different percentages of shell nacre (0.1, 0.2, 0.5, 1, 1.5, 2, 5, 10) to the weight of the precursor 3-TMSPM was taken and the bioresins (BR) were named as tabulated. The siloxane peaks are highly FTIR active and the arrangement of siloxane can be predicted with FTIR analysis. In a polymeric silica network, calcium acts as a network modifier which can affect the molecular weight of the resins[4]. So all the resins were analyzed by refractometry, HPLC, FTIR, GPC and the presence of calcium in BR-1 is further confirmed by OES-ICP.

Sl No	Inorganic content	Amount of inorganic content (%)	Inorganic material used	Code of resin
1	Nil	Nil	Nil	Bare
2	Zr	1	$ZrCl_2$	ZrR1
3	Sr	0.5	$SrCl_2$	Sr-0.5
4	Shell nacre	0.1	$CaCO_3$	BR-0.1
5	Shell nacre	0.2	$CaCO_3$	BR-0.2
6	Shell nacre	0.5	$CaCO_3$	BR-0.5
7	Shell nacre	1	$CaCO_3$	BR-1
8	Shell nacre	1.5	$CaCO_3$	BR-1.5
9	Shell nacre	2	$CaCO_3$	BR-2
10	Shell nacre	5	$CaCO_3$	BR-5
11	Shell nacre	10	$CaCO_3$	BR-10

List of resins synthesized

The highlight of siloxane network is highly thermostable Si-O-Si and the very low glass transition temperature, which was understood by subjecting Bare, BR-1, BR-2, BR-5 and BR-5 to thermogravimetric analysis and differential scanning calorimetry. XRD analysis of bare and BR-1 was done to find the thickness of the siloxane network and arrangement. Later based on yield and transparency, bare resin, BR-1 and BR-2 was taken for further studies. Prediction of the structure of bare resin, BR-1 and BR-2 were done by using ^{13}C and ^{1}H NMR analysis. Structure conformation was observed by TEM analysis.

Preparation of bare, BR-1, BR-2 resin containing bone cements with quartz: Two paste system

Preparation of Paste A:

Bare /BR-1/BR-2 resin - 1 gm
TEGDMA- 1 gm
Silanated quartz 150 parts - 3 gm
Fumed silica (12%)- 0.24 gm
DMAPEA (0.75%) - 0.03gm

Paste A was formulated in the above order and DMAPEA was taken to the total amount of resin mixture (4 gm) and mixed well. Paste was allowed to dissolve for 24 hrs.

Preparation of Paste B

Bare /BR-1/BR-2 resin - 1 gm
TEGDMA- 1 gm
Silanated quartz 150 parts - 3 gm
Fumed silica (12%)- 0.24 gm
BPO (2%) - 0.08gm

Paste B was formulated in the above order and DMAPEA was taken to the total amount of resin mixture (4 gm) and mixed well. Paste was allowed to dissolve for 24 hrs.

Preparation of bone cement

Bone cement consists of liquid component with dissolved 4-(dimethyl amino) phenyl ethyl alcohol (DMAPEA) (Aldrich) (accelerator) and solid component containing filler part (mixture of PMMA +HAP/mixture of PMMA-PS copolymer + HAP) and Benzoyl peroxide (BPO) as the initiator. Several formulations of bone cement were prepared with varying concentrations of filler; accelerator and initiator .Working time and setting time of these formulations were determined

Formulation and physicochemical characterization of bone cement:

Both the paste A & B was mixed well by spatula. Working time and setting time was calculated. Linear polymerization shrinkage and compressive strength of the bone cements was evaluated. *In vitro* bioactivity of the cured samples was studied by soaking the samples in SBF for 0, 1 and 7days. Remineralization was evidenced by SEM-EDS analysis.

Formulation and evaluation of bioactive bone cement with different percentages of shell nacre filler (50 and 300%) with bare, BR-1 and BR-2

Formulated bioactive bone cement with bare resin, bioresin 1 and bioresin 2 using the diluent glycerol dimethacrylate, filler fumed silica (12%) and shell nacre powder (50 parts and 300 parts). Compared the linear polymerization shrinkage, compressive strength and diametral tensile strength of bare, bio- 1 and bio- 2 chemical cured composites and observed bioresin 1(BR-1) exhibited better properties.

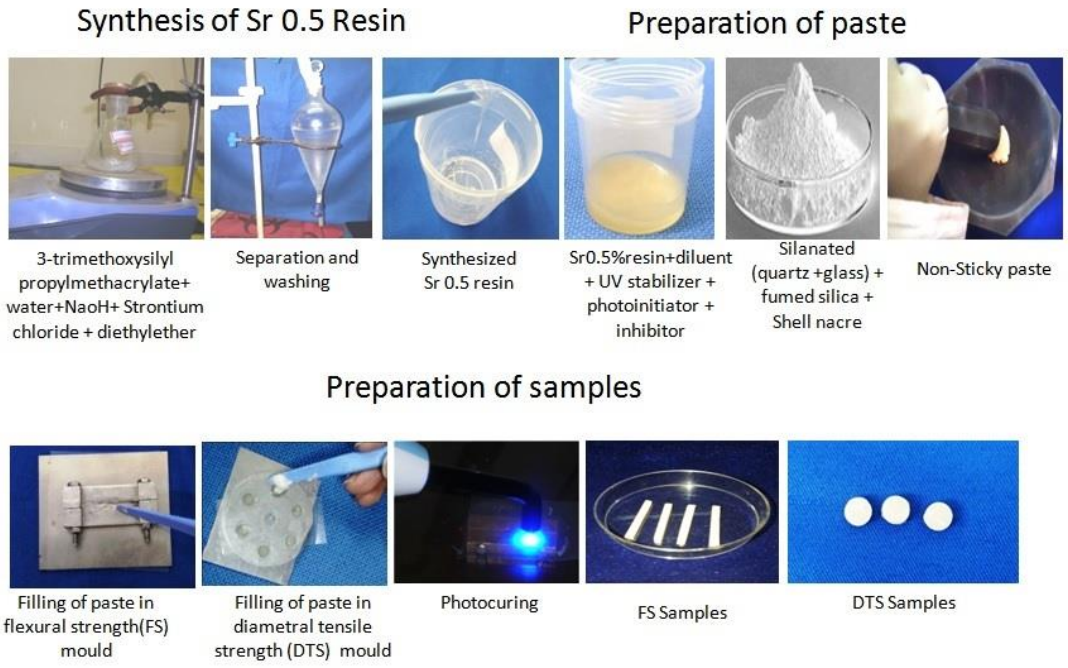
Evaluation of bioactive bone cement with BR-1 and different percentages of shell nacre filler

Bioactive bone cement with BR-1, diluent glycerol dimethacrylate and different amount of shell nacre filler (50,100,150,200,250 and 300 parts) was prepared. Radiopacity was evaluated by using scout images of microcomputed tomography and comparing with Al stepwedge. Linear polymerization shrinkage and porosity (microct) was also studied.

Physicochemical properties of photocured bio-resin bone cements:

Photocured composites (bare resin, bioresin 0.1, bioresin 0.2, bioresin 0.5 and bio-resin 1) were prepared with diluent TEGDMA, photoinitiator and quartz. Studied the linear polymerization shrinkage, depth of cure, compressive strength, diametral tensile strength and flexural strength of all five different sets of resins.

Effect of shell nacre filler on physico-mechanical properties of photocured composites.



Synthesized Sr0.5 resin by modified sol gel method and prepared photocured bioactive bone cements with quartz and glass. Effect of shell nacre (0%, 5%, 10%, 15%, 20%, 25%) filler on physico-chemical properties like linear polymerization shrinkage, volumetric shrinkage, diametral tensile strength, flexural strength of the bioactive bone cement was investigated . Remineralization studies were carried out by soaking the cured cements in SBF for 0,1 and 7 days and observed the hydroxyapatite formation by SEM analysis. Direct contact, MTT and cell adhesion test were done to prove the cytocompatibilty of the cured bioactive bone cement.

Comparison of physicochemical properties of photocure BR-0.1 with Sr 0.5 resin cements

Photocured bioresin 0.1 quartz containing bone cement was compared Sr0.5 quartz cement based on linear polymerization shrinkage and compressive strength. Bioactivity of the bioresin 0.1 composite was examined in SEM after soaking the cured samples for 0,1 and 7 days. Cytotoxicity was evaluated by direct contact, MTT and cell adhesion test.

15. Detailed anaylsis of results :

GPC analysis of ormoresins

GPC analyses of ormoresins were carried out to determine the molecular weight distribution. Results obtained for Ca-0 ALK, Ca-0.2 ALK, Ca-0.25 ALK and Ca-0.5 ALK are given in **Table.**

Table. GPC analysis of alkali hydrolyzed ormoresins

S.No.	Name of resin	Mn	Mw	Mp	Poly dispersity index
1	Ca-0 ALK	3681	5642	4440	1.53
2	Ca-0.2 ALK	3137	4323	3716	1.38
3	Ca-0.25 ALK	8265	54189	13202	6.56
4	Ca-0.5 ALK	6783	17470	11921	2.58

Synthesis and characterization of Hydroxy apatite

Hydroxy apatite was synthesized by two different routes and the particle size was determined .Hydroxy apatite synthesized by route I was optimized since particle size was less, when compared with that synthesized by route II.Hydroxy apatite was sintered at 800°C, 1200°C and 1300°C to determine the effect of sintering temperature on mechanical properties.Hydroxy apatite sintered at 1300°C has shown improved compressive strength when used as filler.Silanation of hydroxy apatite was carried out and compressive strength was determined to study the effect of silanation on mechanical properties. The compressive strength values using silanated HAP and non –silanated HAP indicates that there is statistically no significant result between the two values ($p>0.05$).**Fig.5**.Hydroxy apatite was characterized using FTIR spectroscopy. The major peaks in the spectra are 1051.58cm^{-1},961.34cm^{-1},877.45 cm^{-1},603.61cm^{-1},568.90cm^{-1}, which are characteristic peaks of $PO4^{3-}$ ion.**Fig.3** and **Fig.4**

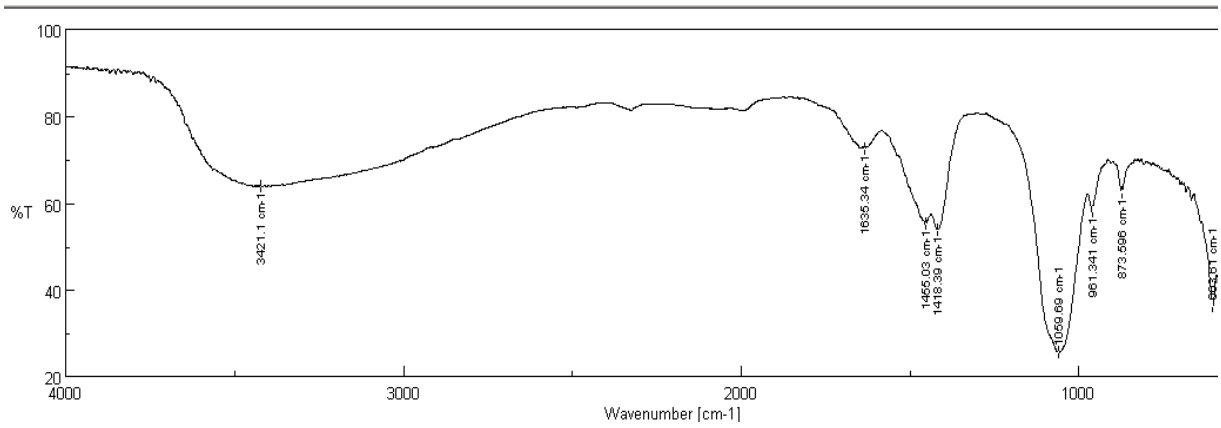


Fig.3 FTIR Spectra of HAP (Route II)

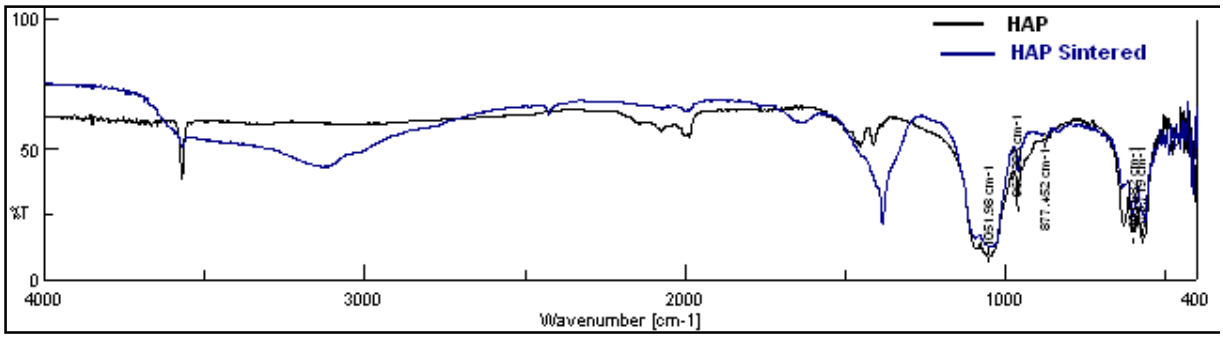


Figure.4 FTIR spectra of HAP (Route I) (sintered & non –sintered)

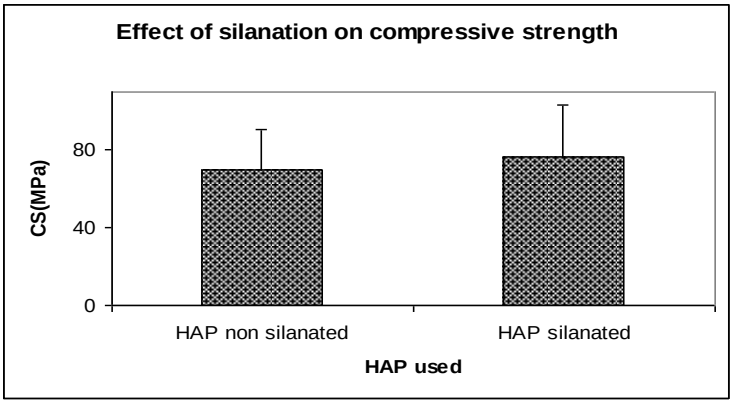


Fig.5 Effect of silanation on compressive strength of bone cement

16. Summary sheet of not more than 2 pages under following heads : (Title, Introduction, Rationale, Objectives, Methodology, Results, Translational Potential)

Title,

Introduction Bone cements are widely used in orthopedic applications to anchor implants to existing bone, reconstruct bone and deliver bioactive agents to the body. Charnley developed the first bone cement in the 1960s using poly (methylmethacrylate) (PMMA), and these materials remain the most widely used method for fixation of prostheses. However, PMMA bone cement cannot bond directly to bone, which occasionally leads to aseptic loosening of prostheses used for arthroplasty. Aseptic loosening is a severe complication in total joint replacement, causing high rates of revision surgery. Bioactive bone cements that can bond directly to bone are promising materials for prevention of this problem. A bioactive bone cement is particularly designed to produce a better interface between the cement and bone.

Rationale

MMA/ PMMA bone cement is beset with a number of drawbacks such as

1. Exothermic temperature of the bone cement rises between 65°C and 125°C leading to the thermal necrosis of the bone, impaired local blood circulation, and predisposition

to membrane formation at the cement-bone interface.

2. The release or leakage of unreacted monomer (MMA) before polymerization of the cement leads to chemical necrosis of the bone.
3. Bone cement shrinkage is another factor contributing to loosening of prostheses.

Objectives,

The aim of the project is to develop bioactive bone cement based on organically modified ceramic resin containing zirconium/calcium/strontium /Shell Nacre as the liquid component and bioactive fillers such as hydroxy apatite along with PMMA or PMMA-PS copolymer as the solid component to overcome the drawbacks of conventional PMMA bone cement.

Methodology.
Described in section 14

Translational Potential: MMA free bone cement has good translational potential after completing further studies.

17. Contributions made towards increasing the state of knowledge in the subject :

The present formulation of bone cement based on the calcium containing resin and combination of HAP/PMMA-co –PS filler is found to have excellent compressive strength, sufficient working time and setting time. Preliminary studies on bioactivity showed that apatite like structure is deposited on the composite during storage in SBF.

18. Conclusions summarising the achievements and indication of scope for future work :

3 patent applications filed . A process for the synthesis of zirconium/calcium/strontium /Shell Nacre containing bio resin for dental and orthopedic application developed. Further studies on in vivo biological and preclinical evaluation, submission of results for publication in high impact journals.

19. Science and Technology benefits accrued :

a. List of research publications with complete details :

CVibha, P.PLizymol*, Synthesis and characterization of a novel radiopaque dimethacrylate zirconium containing pre-polymer for biomedical applications. Materials Letters
https://doi.org/10.1016/j.matlet.2018.11.098

Bridget JW., Susan Mani, Willi ,**Lizymol P.P*.** Synthesis and characterization of ladder structured ormocer resin of siloxane backbone and methacrylate side chain, Materials Letters https://doi.org/10.1016/j.matlet.2021.131192, 310, 131192 ,2022 (Elsevier)

b. Manpower trained on the project :

i.	**Research Scientists or Research Fellows**	**:**	**NIL**
ii.	**No. of PhD's produced**	**:**	**1**
iii.	**Other Technical Personnel trained**	**:**	**1**

c. Patents taken, if any : 3

i. Inventors:,Pampadykandathil Philipose Lizymol , A radiopaque bioactive non cytotoxic bone cement and the process there of Application Number 201941034702 dated 28/08/2019

ii. Inventors:, Pampadykandathil Philipose Lizymol . Bridget W.J., KalliyanaKrishnan V. Indian patent " A process for the synthesis of Shell Nacre containing bio resin for dental and orthopedic application, Indian Patent Application Number 201841028120 dated 26/07/2018, Granted with patent No: 400578 dated 30/06/2022 for 20 years from 26/07/2018

iii. Inventors:,Pampadykandathil Philipose Lizymol . Bridget W.J.Low cost bone cement Application number 202041025478.dated 17/06/2020.Patent title: Low cost bone cement, Granted with patent No. 410827 dated 02/11/2022

d. Products developed, if any : Bone cement formulation

20. Abstract: (In 300 words for possible publication in ..... Bulletin) NA

a. Background:

b. Materials:

c. Results:

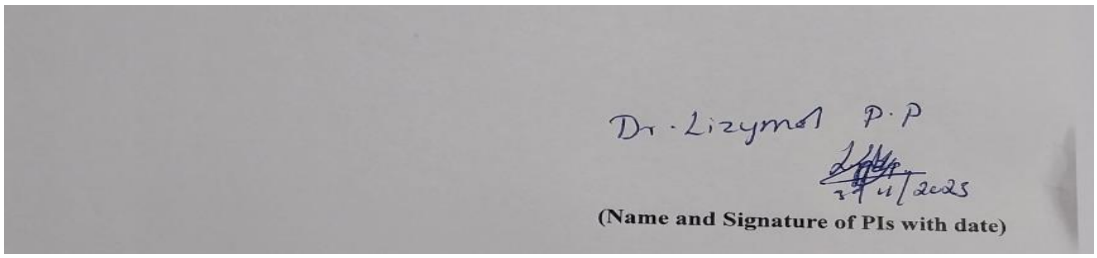
d. Conclusion:

21. Procurement/Usage of Equipment:

a. Details of Equipment: NIL

Sl. No.	Name of Equipment	Make/ Model	Cost (Rs.)	Date of Installation	Utilisation	Remarks regarding maintenance breakdown

b. Suggestions for disposal of equipment(s):Not Applicable



Routing: *Signed copy of "Project completion Report" by PI → root@sctimst.ac.in, rpc@sctimst.ac.in*