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PROJECT COMPLETION REPORT

1. **Project Number** : **6240**

2. **Title of the Project** : Indigenous bone graft expanders for Masquelet Induced Membrane Technique

3. **Funding Agency Name** : TDF-SCTIMST

4. **Project Reference Number provided by the Funding Agency**:IRC No 187

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

6. **Co-Investigators (Name & Address):** 1. Dr.FRANCIS BONIFACE FERNANDEZ, **DBST,BMT W,SCTIMST**

   2.Dr.Anilkumar P.R. BMT Wing,SCTIMST

7. **Implementing Institution** : SCTIMST

8. **Collaborating Institutions** : NIL

9. **Date of Commencement** : 12.11.2020

10. **Duration** : 2years

11. **Date of Completion** : 11.11.2022

12. **Objectives as approved :** This proposal aimed at the development of an import substitute for bone graft expander which is used for the management of infective non-unions, non-unions, tumour resections, compound/ comminuted factures with bone loss by Masquelet's induced membrane technique (MIMT), the best clinical proven methodology which is eradicating amputations.

    i. Synthesis and fabrication of bone graft expanders

    ii. Physicochemical characterization of bone graft expanders

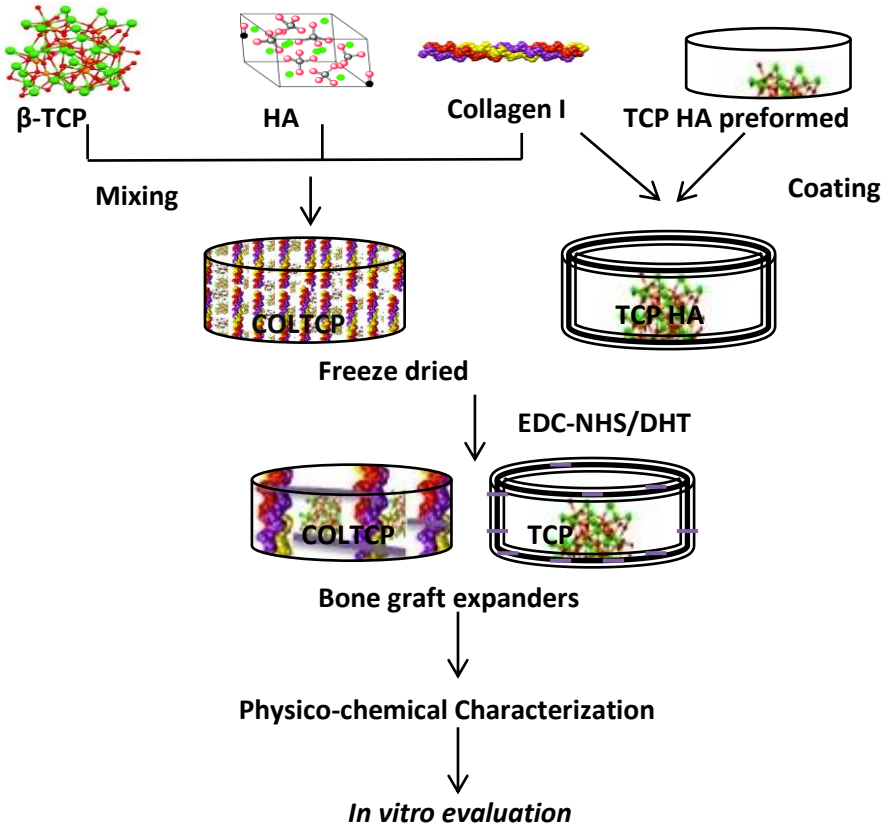
13. **Deviation made from original objectives if any, while implementing the project and reasons thereof : NIL**

14. **Field/Experimental work giving full details of summary of methods adopted, data**

collected supported by necessary tables, charts, diagrams and photographs :

Methodology

The following scheme outlines the methodology of the work. The detailed methodology is as follows:



Scheme I. Methodology of the proposed work**Methodology**

15. Detailed anaylsis of results :

Results, The first initiative in the project is to study the scaffolding properties of Collagen I wet tissue. The collagen wet tissue was soaked in isopropanol and squeezed to remove the isopropanol. And later washed in acetone to remove the water. Further lyophilized and characterized by FTIR.

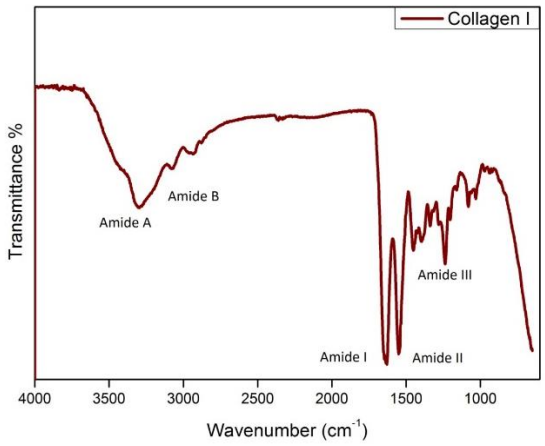


Fig. 1 FTIR spectrum of Collagen I

FTIR analysis revealed the characteristic amide I peak at 1665 $cm^{-1,}$ amide II peak at 1551 cm^{-1} and amide III peak at 1237 cm^{-1} of collagen I. similarly amide A peaks at 3332 cm^{-1} and amide B peak at 2942 cm^{-1} demonstrated the characteristic chemical constituent of collagen. All these peaks confirm that the secondary structure of collagen remained stable irrespective of the process.

HA synthesis was done at bio-ceramics lab. Several batches of HA were synthesized using the salt calcium nitrate tetrahydrate and ammonium dihydrogen orthophosphate by wet precipitation method. After calcination at 300°C, the HA powder was sieved through 125 µ. Synthesized tricalcium powder was provided by bioceramics lab. HA and TCP powder was characterized by FTIR.

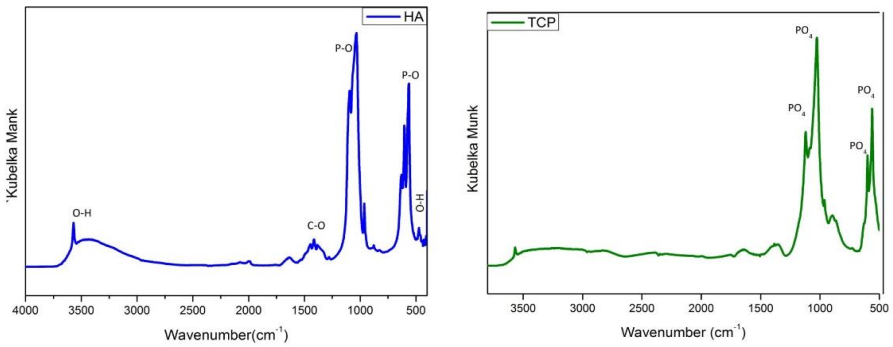


Fig. 2 FTIR spectrum of HA and TCP

FTIR analysis of HA revealed the characteristic HA peaks like OH peaks at 3569 cm^{-1} and 630 cm^{-1}, carbonate peak at 1413 $cm^{-1,}$ phosphate peak at 1033 and 533 cm^{-1}. FTIR analysis of TCP revealed the phosphate peaks at 1120 cm^{-1},1025 cm^{-1}, 599 $cm^{-1}$and 559 cm^{-1}. Thus, both TCP and HA were characterized.

Collagen scaffolding properties were studied with different percentage of collagen I like 1%, 2%, 3%, 4%, 5% and 6%.The scaffolds formed of less than 5% collagen I were very

light and thin whereas scaffolds with 5% and 6% collagen I were better and thicker than the other groups. But the collagen slurries with 6% collagen I were very thick and gel like which reduced the chance of mixing. So, finally 6% collagen I was fixed and different percentages of TCP 10%, 20%, 40% and 60% was added to the 0.05M acetic acid and the pH was adjusted to 3.5. then collagen I was added and stirred at 4°C. After 6 h, composite slurries were poured into moulds, frozen at-80°C and freeze dried. Freeze dried scaffolds are washed well in distilled water and again freeze dried. The obtained scaffolds were shown in fig. 4.

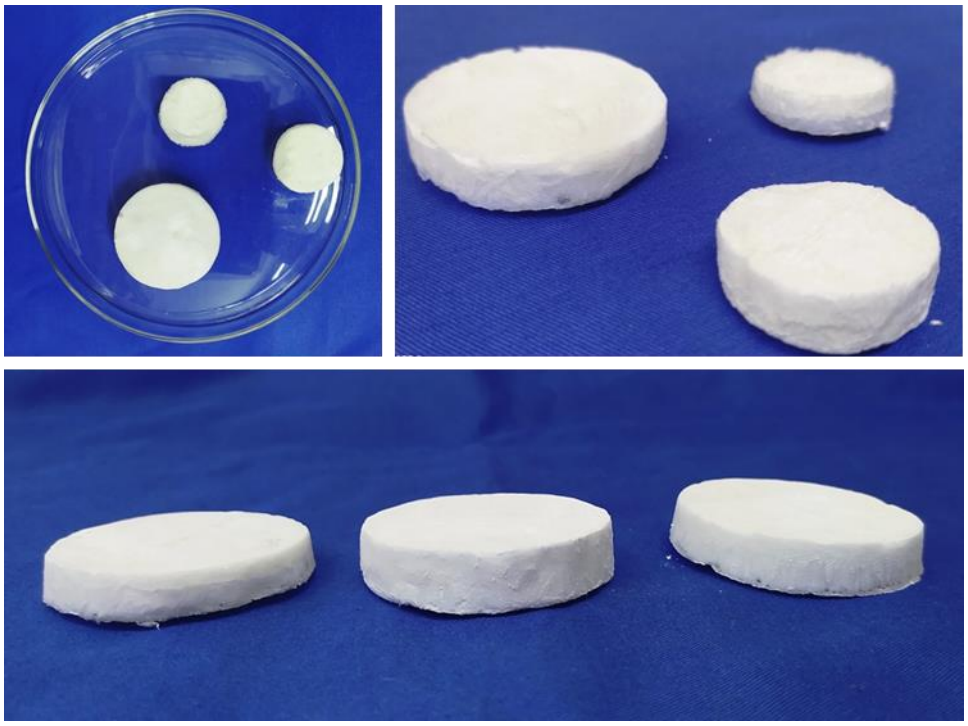


Fig. 4 Fabricated collagen I and Collagen I TCP scaffold

The surface morphology of the scaffolds was studied by SEM analysis. It was observed that both the collagen I (2.5 and 5%) scaffolds had honey comb like structure (Fig.5 and 6) irrespective of amount of collagen I.The morphology of the scaffold is peculiar with line of honey combs arranged parallel to each other.

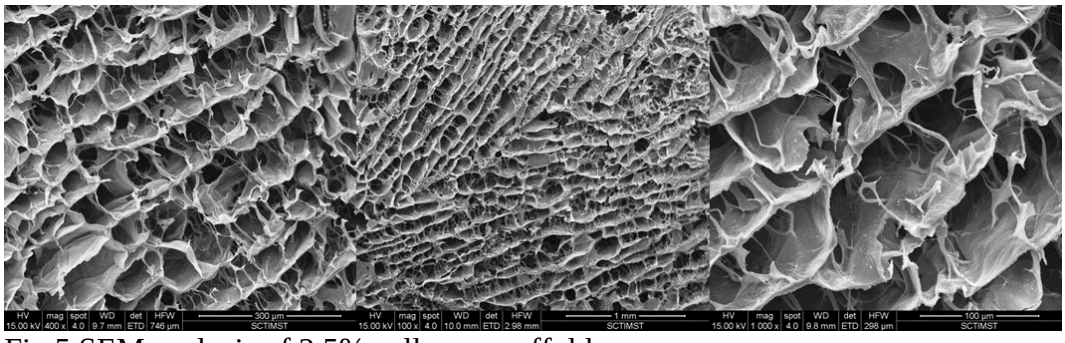


Fig.5 SEM analysis of 2.5% collagen scaffold

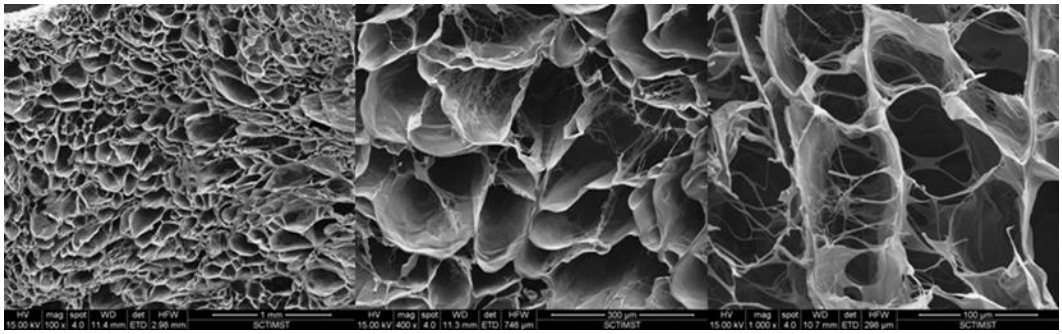


Fig.6 SEM analysis of 5% collagen scaffold

The pores of the 2.5% collagen I scaffolds are nearly 100µm in size but the pore size is not uniform. It exhibited a homogenous distribution of pores like 150 ,200 and 250 µm throughout the scaffold. The architecture of the 5% collagen scaffold (Fig.6) is entirely same with that of 2.5%collagen I.

Microct analysis of the collagen revealed the percentage porosity of both the scaffolds. Collagen I (2.5%) was69% porous whereas 5% collagen was of 63% porous. The parallelly arranged structures (Fig. 7) were observed in microct analysis also. The scaffold was porous throughout the entire structure with heterogen

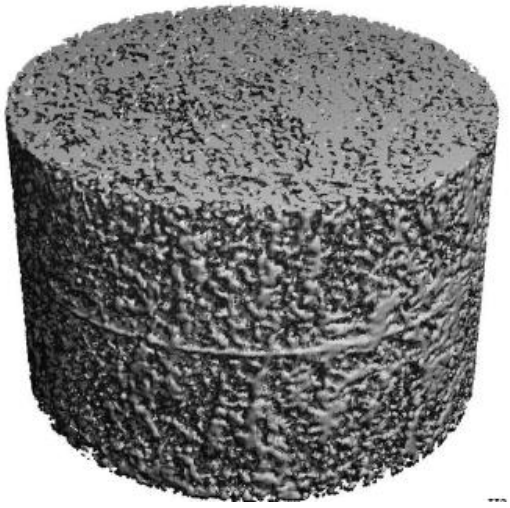


Fig.7Microct analysis of 5% collagen scaffold.

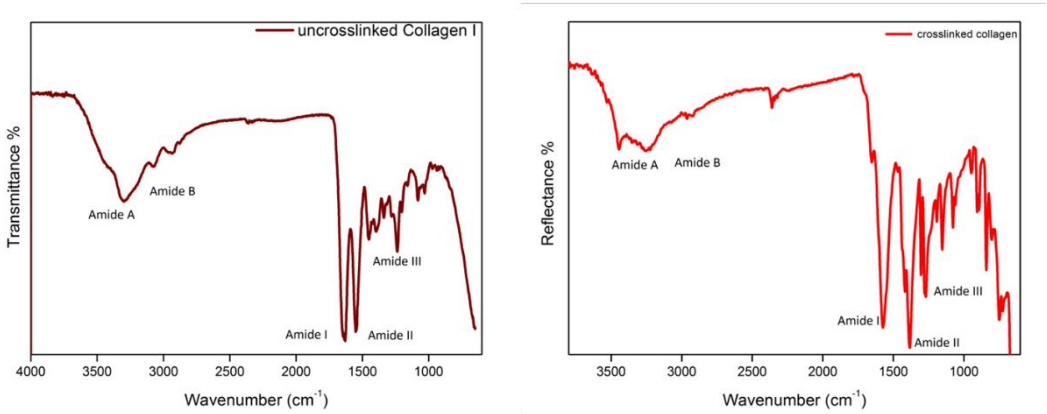
Freeze dried scaffold were soaked in 50 mM EDC/25 mM NHS solution and crosslinked.

After crosslinking, the scaffolds were washed well in deionized water and freeze dried. The intra and inter cross-linking of collagen I was understood by FTIR analysis. Intense amide peaks of A, B, I II and III of crosslinked scaffolds proved the crosslinking of collagen I (Fig.8). The crosslinking of the scaffold enhanced the amide bonding both intra and inter

in the collagen helix.

Fig.8 FTIR analysis of crosslinked and uncross linked collagen scaffolds

Crosslinked scaffolds exhibited higher tensile strength than uncross linked scaffolds. The intense intra and inter amide bonding in the collagen increased the tensile strength of scaffold. Similarly, the modulus of the crosslinked scaffold is significantly higher than uncrosslinked scaffolds.



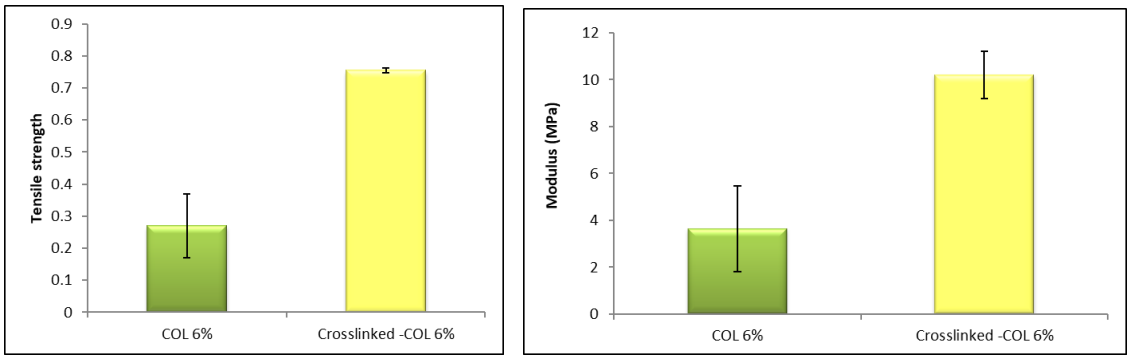


Fig.9Tensile strength of crosslinked anduncross linked collagen scaffolds

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

Title, Indigenous bone graft expanders for Masquelet Induced Membrane Technique

Introduction This proposal aims at the development of an import substitute for bone graft

expander which is used for the management of infective non-unions /non-unions, tumour resections, compound/ comminuted factures with bone loss by Masquelet's induced membrane technique (MIMT), the best clinical proven methodology which is eradicating amputations.

Rationale

Bone is the second most frequent transplanted tissue. The bone loss can be structural defect (osteoporosis, osteoarthritis, metabolic diseases *etc*), primary loss (open fractures, gunshot wounds, osteoclastic tumours) or secondary loss (tumour resection, infection, surgeries and non unions). The major orthopaedic reconstructive challenges are treatment of traumatic bone defects or surgical debridement due to non-unions, tumour, infections, joint fusion or congenital pseudarthrosis. India lacks the uniform standard of health care throughout the country. The real scenario is traditional bone setters treat 60% of orthopaedic issues and complicate the situation to mal-union, non-union, or an established infected non-union following an open fracture. Hospitals treat a tremendous number of the above non-unions than fresh factures. This reflects in the report of 0.5 million amputations with an increase of over 23,000 every year (https://innovate.mygov.in/wp-content/uploads/2018/08/mygov1534863628147178.pdf).

Ilizarov's bone transport, vascularised bone graft and Masquelet induced membrane technique (MIMT) are the different segmental defect management strategies in long bones. Out of all these methods, the French surgeon's Masquelet technique is simple, easy to perform and recorded high success rate. This MIMT is effective in managing defects >2 cm with low rate of complications. The average time required to heal 1 cm defect was 1.24 months. Recently El Jawahari compared Orthoss Collagen (xenograft with a combination of orthoss bovine HA and 10% porcine collagen, Geistlich, Wolhusen, Switzerland)and Vitoss (synthetic β –tricalcium phosphate scaffold with bovine collagen I, Stryker, Malvern, PA) with Orthoss and found that collagen containing scaffolds promoted the attachment and proliferation of BM MSCs and performed superior when compared to orthoss. So a composite scaffold of collagen, HA, TCP and may evolve as a promising bone graft expander equivalent to international standard and meet the urge need of orthopaedics.

So it is hypothesized that ***composite scaffold with collagen, tricalcium phosphate and hydroxyapatite will be a suitable bone graft expander for segmental defect management by MIMT.***

Objectives,

i. Synthesis and fabrication of bone graft expanders

ii. Physicochemical characterization of bone graft expanders

Methodology,

1. **Fabrication of bone graft expanders:**
   a. **Characterization of collagen I:**

      Collagen I (biomedical grade) was purchased from Cologenesis as wet tissue soaked in isopropanol. The collagen wet tissue was squeezed and washed in acetone. Further lyophilized and characterized by FTIR

   b. **Synthesis and characterization of HA and TCP:**

      HA synthesis was done at bio-ceramics lab. Several batches of HA were synthesized by wetprecipitation method. After calcination at 300°C, the HA powder was sieved through 125 µ. HA powder was characterized by FTIR. TCP was provided by bio-ceramics lab, which was further characterized by FTIR.

   c. **Fabrication of scaffolds:**

      Collagen scaffolding properties were studied with different percentage of collagen I like 1%, 2%, 3%, 4%, 5% and 6%. Collagen slurries were prepared using 0.05M acetic acid at 4°C for 6 h. Then poured into moulds, frozen at -80°C and then freeze dried. Composite collagen scaffolds (COLTCPHA)with different amount of TCP 10%, 20%, 40% and 60% was fabricated. First 0.05M cold acetic acid was prepared and TCP was stirred well overnight. After adjusting pH, 6% collagen was added, stirred well for 6h and then poured into moulds, frozen at-80°C and freeze dried. Similarly preformed scaffolds were extruded using 12% PVA HA suspension (M.W. 12,000) and porous HA scaffold were formed by calcinating at 300°C.

   d. **Cross-linking of collagen I**:

      Collagen I was cross-linked by immersion in an EDC/NHS solution (N-(3-Dimethylaminopropyl)-N'ethylcarbodiimidehydrochloride/N hydroxysuccinimide.The samples were then washed in deionizedwater to remove the unreacted EDC/NHS solution for 24 hwith water change of every 4 h. Finally, the samples were freeze-dried to obtain porous scaffolds.

2. **Physicochemical characterization of bone graft expanders**
   a. **Architectural analysis of scaffold**

      Scaffold morphology, pore size, percentage of porosity and interconnected scaffolds was evaluated by microcomputed tomography and

SEM analysis.

b. Chemical investigation

Chemical composition of the scaffolds was confirmed by FTIR XRD and thermogravimetric analysis.

Translational Potential: A proof of concept was established for the indigenous bone graft expanders. This will form the basis for the further preclinical and clinical studies with a target of import substitute for bone graft expanders.

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

EDC/NHS solution (N-(3-Dimethylaminopropyl)-N'ethylcarbodiimidehydrochloride /N hydroxysuccinimide crosslinked Collagen I scaffolds exhibited higher tensile strength than uncross linked scaffolds. The intense intra and inter amide bonding in the collagen increased the tensile strength of scaffold. Similarly, the modulus of the crosslinked scaffold is significantly higher than uncrosslinked scaffolds.

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

Collagen I (biomedical grade) as wet tissue was purchased from Cologenesis, Salem, which was characterized by FTIR to confirm the chemical constituent. Tricalcium phosphate was provided by bio-ceramics laboratory. Hydroxyapatite was synthesized by wet precipitation method and characterized. Scaffolding of collagen was tried with different percentages of collagen I like 1%, 2%,3%,4%, 5% and 6%. Similarly, collagen scaffold was crosslinked by EDC-NHS method and the crosslinking was confirmed by FTIR analysis. Composite scaffolds of collagen I and TCP was fabricated with 10%, 20%, 40% and 60% of TCP and 5%of Collagen I by freeze dry method and characterized. Similarly preformed TCP HA scaffolds were fabricated in collaboration with bio-ceramics lab. Thus, a 3D porous scaffolds of TCP, HA and collagen was fabricated, characterized and a proof of concept was established.

18. Science and Technology benefits accrued :

a. List of research publications with complete details : NIL

b. Manpower trained on the project :

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

c. **Patents taken, if any : NIL**

d. **Products developed, if any : 1 proof of concept**

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

Dr. Lizymol P.P

27/11/2025

(Name and Signature of PIs with date)

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