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PROJECT COMPLETION REPORT

1. **Project Number** : **TRC Project – P 8169**

2. **Title of the Project** : **Preparation and standardization of a batch of Reference Biomaterials (RM) for biological evaluations**

3. **Funding Agency Name** : **Technical Research Centre, DST**

4. **Project Reference Number provided by the Funding Agency**: **P 8169**

5. **Principal Investigator (Name & Address) :** Leena Joseph, BMT Wing-SCTIMST

6. **Co-Investigators (Name & Address):**
   i. Dr. Ramesh P (Co-PI) (preparation of polymeric materials)
   ii. Dr. Remya NS(I)(toxicological evaluation)
   iii. Ramesh Babu(I)(fabrication of sample)
   iv. Dr. Anil Kumar PR (I) (cytotoxicity evaluations)
   v. Dr. Anugya Bhatt (I) (blood compatibility evaluation)
   vi. Dr. Sabareeswaran A (I) (Histopathology evaluation)

7. **Implementing Institution** : BMT Wing-SCTIMST

8. **Collaborating Institutions** :-

9. **Date of Commencement** : 16/09/2017

10. **Duration** : 3 years 3 months (approved period)

11. **Date of Completion** : 27 Nov 2020(RM for Cytotoxicity studies)

12. **Objectives as approved :**
    1. Identification & specification development of candidate materials for development as RMs in the selected areas of biomaterial evaluation.
       - Muscle and bone Implantation studies (RM type-**metal, polymer & ceramic**)
       - Cytotoxicity studies (RM type-positive & negative controls)
    2. Standardize procedures for RM production.

3. Establish the characteristics / properties by means of physic-chemical / biological evaluations.
4. Production of one pilot batch (typically suitable for 1000 tests) in each category.

13. Deviation made from original objectives if any, while implementing the project and reasons thereof : RM for Muscle and bone Implantation studies is completed only on June 2023 hence publishing of results etc.; are in ongoing stage.

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

Summary: The Biomedical Technology Wing has implemented a quality system meeting international standard ISO/IEC 17025 and is accredited by Le Comite Francais d'Acreditation (COFRAC), France. As part of establishing traceability of ISO 17025 accredited tests, an in-house calibration facility is operational since the year 2000 (NABL accredited from the year 2010). Wherever traceability establishment is impossible with calibrations, the use of RMs is the recommended solution.The CRMs(Certified Reference Material) available for biological evaluations are only a few (not indigenously available) and need to be imported at a high cost. In regular practice, RMs are prepared in-house at SCTIMST by comparing their property value with imported CRMs for biological evaluations as experimental controls.

A proof of concept project(TRC) was executed for the development of Reference Materials (RMs) for the biological evaluation of medical devices. A batch of RMs was prepared for the in-house use .

1 Materials and Methods

1.1 Materials

The PVC RM material was prepared by mixing PVC and relevant additives. plasticizer is added, to make the material flexible. Appropriate stabilizer was used to prevent the thermal degradation.

1.2 Synthesis and characterization

RM has to undergo different processing steps for making it suitable for using in cytotoxicity evaluations.

a) Qualitative analysis for verification of material identity

b) samples are molded to desired shape at suitable temperatures

d) Aseptic cleaning and packaging

e) Sterilization

i) Safety precautions by assuring safe limits of toxic residues from sterilization gases.

1.2.1 Synthesis of positive control

Dry blending and melt mixing was done at suitable temperature as the preliminary step in the synthesis [M.O. Rodrigues,et al.Impacts of plastic products used in daily life on the environment and human health: What is known?,Environmental Toxicology and Pharmacology,Volume 72,2019,103239,ISSN 1382-6689,https://doi.org/10.1016/j.etap.2019.103239.]. From PVC sheets circular discs were prepared using stainless-steel dies.

1.2.2 Packaging and Sterilization

The samples were cleaned using mild detergent and distilled water in an ultrasonic cleaning machine. Polymer samples were dried over night in an oven . The samples were then randomized before packing for distribution. Packaging was

carried out in clean area. The packets were then ethylene oxide (EO) sterilized. Storage and handling instructions of samples were provided in outer package labels

1.2.3 Contact angle measurement

Contact angle, θ (theta) measurement, is a quantification of wetting of a solid by a liquid. The contact angle is geometrically described as the angle formed by a liquid at the three-phase boundary where a liquid, gas, and solid intersect.

1.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR(Nicolet 5700 Thermo Fisher Scientific, USA)spectrum of the material is considered as molecular fingerprint, and used to confirm the identity of PVC-RM samples.

1.2.5 Thermal analysis- Differential Scanning Calorimetry.

Heat capacity behaviour of RM samples was done in A Differential Scanning Calorimeter (DSC Q100, TA instruments Inc).

1.2.6 Thermal analysis- Thermo gravimetric analysis

Understanding the thermal stability was done using SDT Q600 V8.3 .Reference material used for the analysis was Calcinated Alumina.

1.2.7 Surface roughness

The surface finish of the PVC samples was studied using a non contact profilometer (Taylor Hobson, UK). Line profile of total six number of samples was taken with the purpose of understanding the nature of surface finish.

1.2.8 Tensile strength

RM samples were studied to understand thetensile characteristicsas part of mechanical characterisation studies. The instrument used was Universal Testing Machine .

1.3 Biological evaluation

1.3.1 Cell culture

Cytotoxicity of the material was evaluated according to the ISO 10993-5 standard: Test by Direct Contact Method. The negative control used was Ultra High Molecular Weight Polyethylene (UHMWPE) and Positive control was confirmed stabilized polyvinyl chloride (PVC).

1.3.2 Cytotoxicity evaluation

The reactivity of samples were graded as 0, 1, 2, 3 and 4 based on zone of lysis, vacuolization, detachment and membrane disintegrationas per the specifications in ISO-10993-5 Standard as shown in the table below.

Table 1: Reactivity grades in assessing cytotoxicity

Grade	Reactivity	Description of reactivity zone for sample with 1 cm diameter
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen

2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1 cm
4	Severe	Zone extending farther than 1 cm beyond specimen

1.4 Inter-Laboratory comparison (ILC)

The reproducibility to induce cytotoxic response of PVC was tested by inter-laboratory comparison. The samples were submitted to another laboratory who performed the same test according to ISO 10993: Biological Evaluation of Medical Devices, Part 5: Tests for cytotoxicity: in vitro Methods. The author's laboratory initiated the ILC and the samples were coded to blind the identity.The reactivity was graded as 0, 1, 2, 3 and 4 on the basis of - zone of lysis, vacuolization, detachment and membrane disintegration.The results from participating laboratories were compared for the similarity in grading as 0, 1, 2, 3 and 4 as per ISO 10993-5.

2 Results

2.1.1 A reference material facility is established in the division.

A batch of RMs was prepared for the in-house use .

- Muscle and bone Implantation studies (RM type-**metal, polymer & ceramic**)
- Cytotoxicity studies (RM type-positive & negative controls)

2.2 Synthesis and characterization

2.2.1 PVC based RM was prepared by compounding with suitable chemicals to provide desired cell reactivity performance in invitro evaluation of biomaterials. Characteristics of physico chemical and biological property values were then established through a series of evaluations.

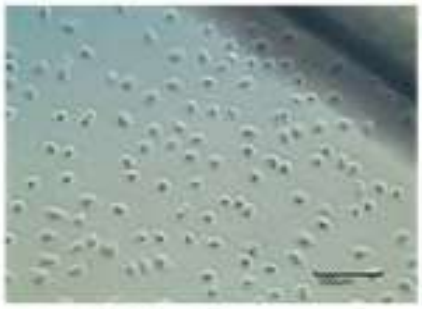

Fig: L929 cells around PVC based RM material with positve reactivity.

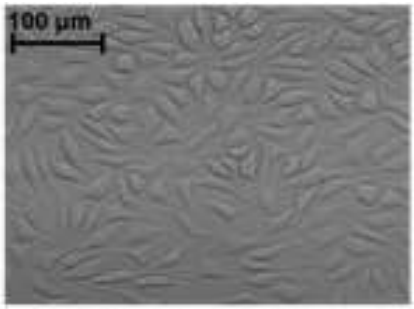

Fig : With Negative control ; Viable cells in the proximity of sample.

2.2.2 PVC base Rm in its package is indicated in figure below. [Remya, N.S., Joseph, L. (2022). Certified Reference Material for Qualifying Biomaterials in Biological Evaluations. In: Aswal, D.K., Yadav, S., Takatsuji, T., Rachakonda, P., Kumar, H. (eds) Handbook of Metrology and Applications. Springer, Singapore. https://doi.org/10.1007/978-981-19-1550-5_32-]

PVC Polymer

Figure 3 An example of polymer reference material (Positive control material made of PVC)

15. Detailed anaylsis of results :

Detailed analysis of results is under the consideration for publishing.

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

As in section 14

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

The publications in this segment invited attention of National Physical Laboratory(CSIR-NPL) which is the national APEX authority in metrology to make a visit at SCTIMST. Feasibility of bringing these indigenously in-house developed RMs under the national umbrella of the Bharatiya Nirdeshak Dravya (Indian Reference Material)program of the National Physical Laboratory(CSIR-NPL) was discussed in their meeting during visit by Head-BND Management & Technical Coordinator, and Head-BND Outreach & BND Division, CSIR-NPL during January 2023.

The visit concluded with following recommendations

- Collaborative programme between NPL and SCTIMST on RM Development is feasible
    - RM development will be the responsibility of SCTIMST
    - Establishing traceability will be done by CSIR-NPL
- Feasibility to transfer Technology to ISO 17034 accredited RM producer will be explored.

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

- A batch of RMS in 3 classes(Metal, Ceramic & Polymer) is prepare and in routine use. Annually 700 RM samples are used in different product development projects and research (Total 2100 RM samples were issued for last 3 years which may cost Rs.4,20,000 as per internal costing).
    - Muscle and bone Implantation studies (RM type-**metal, polymer & ceramic**)
    - Cytotoxicity studies (RM type-positive & negative controls)
- Unless in-house RMs were not prepared, we would have to spend 5 to 8 times more expenses on import purchase for RMs.
- Publications: 7 No.s(3 articles, 1 book chapter& 3 conference proceedings)
- **scope for future work**

  National need for RMs for biological evaluation of medical devices is identified by the research team at NPL, India . A Joint project proposal for a collaborative program can lead to a national program addressing national/international

market requirement for RM products. This can replace imports of CRMs(certified reference Material) from Unites states, Japan in due course.

19. Science and Technology benefits accrued :

a. List of research publications with complete details :

1. Significance of Metrological Tools in an ISO 17025 Accredited Quality System for a Biological Evaluation Facility. MAPAN 37, 683?691 (2022). https://doi.org/10.1007/s12647-021-00517-2, **MAPAN-Journal of Metrology Society of India**, 2022
2. Certified Reference Materials for Scientific and Industrial Applications. *MAPAN* **37**, 465–467 (2022). https://doi.org/10.1007/s12647-022-00604-y
3. Availability of Reference Materials for Improving Quality of Life within Scientific and Industrial Framework. ijetms;7(5):212-218
4. Remya, N.S., Joseph, L. (2022). Certified Reference Material for Qualifying Biomaterials in Biological Evaluations. In: Aswal, D.K., Yadav, S., Takatsuji, T., Rachakonda, P., Kumar, H. (eds) Handbook of Metrology and Applications. Springer, Singapore. https://doi.org/10.1007/978-981-19-1550-5_32-1
5. 'Standardization of PVC based (Tin Compound Stabilized) Positive Control – Reference Material for Cytotoxicity Evaluation'. Proceedings of World Biomaterial Congress, Glasgow: WBC2020-LATE-4419
6. 'On the development of Control Materials for Biological Evaluations', Proceedings of International Conference on Recent Trends in Materials Science and Technology 2018 (ICMST 2018) , 2018 ,Page 1.36 to 1.37
7. Tin compound stabilized Polyvinyl Chloride as Positive Control for Cytotoxicity Evaluations', Proceedings of International Conference on Recent Trends in Materials Science and Technology 2018 (ICMST 2018) , 2018 ,Page 2.140 to 2.14

b. Manpower trained on the project :

i.	**Research Scientists or Research Fellows**	**:**	**1(Engineer)**
ii.	**No. of PhD's produced**	**:**	**nil**
iii.	**Other Technical Personnel trained**	**:**	**nil**
c.	**Patents taken, if any**	**:**	**nil**
d.	**Products developed, if any**	**:**	**RM** for cytotoxicity(positive & negative control) RM for implantation studies(Muscle& Bone**)**

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

a. Background: With the anticipated increase in demand for biomaterial & device qualification in medical devices development; preclinical biological evaluation under regulatory platforms is essential. Certified reference materials are the essential metrological tools in establishing reliable and accurate evaluation results for qualifying regulatory requirements in par with international excellence. Currently Certified RMs by imports are available in market. But affording costlier RMs is not always feasible and hence need for indigenous RMs to meet the role of imported RMs is identified. By pursuing the RM development project, SCTIMST has established a methodology and

established a RM facility for preparation of RMs for the use in cytotoxicity evaluations, bone- muscle Implantation studies according to International standards.

b. Materials:

Polymer, Metallic& Ceramic raw materials

c. Results:

- A batch of RMS in 3 classes(Metal, Ceramic & Polymer) is prepared and in routine use. Annually 700 RM samples are used in different product development projects and research (Total 2100 RM samples were issued for last 3 years which may cost Rs.4,20,000 as per internal costing).Unless in-house RMs were not prepared, we would have to spend 5 to 8 times more expenses on import purchase for RMs.

d. **Conclusion:** When RMs prepared at SCTIMST with a medical device development background, are made traceable to SI units via NPLI ; they will be designated as BND(Bharatiya Nirdeshak Dravya) or IRM(Indian Reference Material). . Also the availability of SI traceable BNDs® (Bharatiya Nirdeshak Dravya) are poised to boost the "Make in India" program along with the harmonization of the quality infrastructure of the country. Feasibility to transfer Technology to ISO 17034 accredited RM producer will be explored.

21. Procurement/Usage of Equipment:

a. Details of Equipment:

Sl. No.	Name of Equipment	Make/ Model	Cost in Lakhs (Rs.)	Date of Installation	Utilisation	Remarks regarding maintenance breakdown
1	Specimen cutting/grinding machine	Vetex	2.883	19-Nov-19	Regular use	Nil
2	Wire Polishing machine	Bharath Machinery & spares, Mumbai	3.48	31-Dec-19		
3	Electronic Balance that can be used with density kits	Sartorius	2.72	9-Jan-18		
4	Labelling Machine	Brother	0.135	28-Jan-19		

b. Suggestions for disposal of equipment(s):

Under use for RM preparation

Leena Joseph,
Calibration Cell

(Name and Signature of PIs with date)

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