PROFORMA FOR SUBMISSION OF PROGRESS REPORTS OF PROJECTS SUPPORTED UNDER THE SCHEME

INSTRUCTIONS FOR SUBMISSION OF PROGRESS REPORT

Project report should be neatly typed (in single spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset process. Coloured Photographs (4-5 good action photographs), tables and graphs should be accommodated within the manuscript or should be enclosed with captions. Sketches and diagrammatic illustrations may also be given giving step by step details about the methodology followed in technology development/modulation, transfer and training. Any correction or rewriting should be avoided.

Please give information under each head in serial order. Typing should be done in the specified area.

Please do not leave any item unanswered.

Note: Slides, charts, photographs, training manual (with details contents of training programme technical details and techniques involved) or any such display material related to project activities should be brought at the venue of the group monitoring workshop.

SCIENCE FOR EQUITY EMPOWERMENT & DEVELOPMENT DIVISION
DEPARTMENT OF SCIENCE & TECHNOLOGY
TECHNOLOGY BHAVAN
NEW DELHI - 110 016

1. DST's NUMBER & TITLE OF THE PROJECT

DST Number : SSD/WS/070/2011

Project Title: Tissue-engineered ceramic for promoting osteointegration in osteoporotic animal models with relevance to the clinical problem in women.

2. PI & CO-PI's NAME & ORGANIZATION (Complete address, phone no., fax, e-mail):

Principal Investigator :

Dr. Annie John (Rtd, hence charge handed over to Dr. Varma)

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3. SUMMARY OF PROGRESS MADE SO FAR (Only salient features – point wise:)

- Selection of material of choice of study for osteoporotic applications– Hydroxyapatite (HA-Control), Silica coated Hyroxyapatite (HASi- Test 1) and Strontium incorporated hydroxyapatite (SrHA- Test 2)

- Physiochemical characterization and cytocompatibility assessment of control and test materials.
- Fabrication of Tissue engineered ceramic constructs - cHA, cHASi and cSrHA using Mesenchymal Stem Cells (MSCs) and cytocompatibility of the scaffolds were proved.
- Rat osteoporotic model developed by ovariectomy, Calcium deficient diet and model validated using micro CT.
- *In vitro* osteogenic efficacy of in house developed scaffolds – cHA, cHASi and cSrHA were proved using rat bone marrow derived MSCs (rBMSCs).
- Osteogenic efficacy of scaffold of choice to heal critical-sized femur 'drill hole' defects (3 x 1.5 mm) was proved by 6 weeks implantation studies in rat osteoporotic model.

4. DATE OF START: 13.5.2015

SCHEDULED DATE OF COMPLETION: 13.5.2018

5. TOTAL SANCTIONED COST OF THE PROJECT: Rs. 34,94,916/-

TOTAL EXPENDITURE: Rs. 6,87,300/- (actual) + Rs. 7,43,551/- (committed) = Rs. 14,30,851/- as on 31-03-2016

6. INTRODUCTION (Need Assessment For S & T Intervention In Project Area):

Osteoporosis has become a major health problem worldwide especially among the aged population and post-menopausal women. It is defined as a skeletal metabolic disease resulting in fragile bones with low bone density and increased fracture susceptibility. Reported incident rate in India is 20% in women and 15% in men above 50 years (Indian Medical Survey 2015). 80% of the aged population in Kerala is estimated to have a low bone density and together with their increased life expectancy and genetic pre-disposition of low vitamin D level, there is an increased probability to succumb to more incidences of osteoporotic fractures (Babu et al., 2015). Osteoporotic fractures are often associated with significant morbidity, disability, decreased quality of life and mortality in severe cases. The increased risk of fracture and the compromised fracture healing ability, together makes osteoporotic fracture fixation an orthopaedic challenge in the health care system.

Innovation of technology for osteointegrative implants and implant stabilization techniques may improve the existing bone fracture fixation strategies. Strontium ions (Sr) have been proven effective for osteoporotic applications as it can increase the activity of osteoblast cells and simultaneously decrease the activity of osteoclast cells (Canalis et al., 1996) and this has been applied in osteoporotic drugs like Strontium ranalate. Also in-house developed hydroxyapatite (HA) and Silica coated HA (HASi) scaffolds has exhibited improved bone regeneration ability in healthy small and large animal models. Therefore evaluation of the influence of Sr or Si incorporated HA scaffolds for osteoporotic applications has important implications. In the present study, a tissue-engineering (TE) strategy has been adopted, wherein the scaffolds has been fabricated into functional implants by loading osteogenic-induced MSCs *in vitro* and evaluating them further in osteoporotic animal models for its efficacy in bone healing.

7. APPROVED OBJECTIVES OF THE PROJECT:

A. Fabrication of tissue engineered bio-active Scaffold:

B. Osteoporotic model development – Rat

C. *In vivo* evaluation of bio-active scaffold in rat osteoporotic model.

D. Osteoporotic model development – Sheep

E. *In vivo* evaluation of bio-active scaffold in sheep osteoporotic model.

F. To extrapolate data to real clinical situations in women.

8. PROJECT AREA & AREA COVERED (Block, Village & Total Area Covered):

9. COMMUNITY BACKGROUND: NA

10. DETAILED PROGRESS REPORT :

10.A. METHODOLOGY & SYSTEMS APPROACH ADOPTED : (Survey; PRA Exercise; Community Mobilization & Social Engineering; Technology Identification, Modulation & Diffusion; Demonstration & Training Component, Objective wise achievements etc.)

Progress report – (13.5.15 till 31.12.16)

Research methodology used:

A. **Material synthesis and physiochemical characterization:**

Scaffold of choice were synthesized by wet precipitation method (Chandran et al., 2016). Briefly HA powder was prepared by adding stoichiometric amount of ammonium dihydrogen orthophosphate solution into ammoniated aqueous solution of calcium nitrate. SrHA powder was prepared by the slow addition of aqueous ammoniated solution of ammonium dihydrogen orthophosphate into the aqueous solution mixture containing ammoniated calcium nitrate and Strontium Nitrate in the 9:1 mole ratio. HA was dip coated in silica sol to synthesize HASi. All test and control materials were utltra-sonicated and autoclaved by steam sterilization prior to *in vitro* and *in vivo* studies.Phase analyses was determined using X-ray powder diffraction (XRD) and *in vitro* cytotoxicity was evaluated using test on extract method using L929 cells.

B. **Fabrication of tissue engineered scaffolds using rat derived MSCs:**

Rat bone marrow derived Mesenchymal Stem Cells (rBMSCs) were isolated from femoral bones of Wistar rat and cultured in a cell culture incubator at 37°C in a humidified atmosphere of 5% v/v CO2. Isolated cells were characterized for mesenchymal origin using plastic adherence and flow cytometry. Cells in P3 passage were induced towards osteogenic lineage and then seeded onto pre-conditioned scaffolds – HA, HASi and SrHA to fabricate cell-ceramic construct – cHA, cHASi and cSrHA. Direct contact assay was done to evaluate the cytocompatibility of the cell seeded scaffold. To assess cell adhesion ability of the scaffolds, the cell seeded scaffolds were stained with DAPI and then observed under Fluoresence microscope Leica DM 6000. *In vitro* osteogenic efficacy assessment was done using Alkaline Phosphatase Assay (ALP).

C. **Development and validation of rat Osteoporotic model:**

Rat osteoporotic models were developed by bilateral ovariectomy. Briefly, rats were anaesthetized, a small single longitudinal skin incision was made on both sides of the mid-ventral aspect. The ovaries were exposed, ligated and ovary was removed. Animals were given antibiotics and analgesics during post operative period. Animal experiments were conducted as per the guidelines and approval of CPCSEA and Institute Animal Ethics Committee (IAEC). Osteoporosis induced rat model was evaluated using micro CT post 10 months of induction.

D. *In vitro* osteogenic efficacy assessment of the scaffold of choice:

Osteogenic efficacy of the ceramic material (bare and cell seeded) to repair and regenerate bone defects in osteoporotic animals was evaluated using rat models. 3mm bone defects were made in the distal femur of osteoporotic rat model and evaluated post 8 weeks of implantation using histology and histomorphometry.

10.B. TECHNICAL BACK-UP SUPPORT & LINKAGES WITH NEARBY INSTITUTIONS:

All technical evaluations, experimental set up including animal experimentation were all carried out in the host institute (SCTIMST) itself, as we have a well established infrastructure for assessing in house developed materials indented for clinical applications. Surgical procedures for developing rat osteoporotic model in the proposed study was done by Dr. Harikrishnan, who is one of the co-investigators of the project.

10.C. SCIENCE & TECHNOLOGY COMPONENT: TECHNOLOGY PACKAGE DEVELOPMENT-NEW INNOVATIONS/OBSERVATIONS:

A. **Physiochemical characterization of material of choices:**

Phase analysis done using XRD (figure1) indicated the respective phases in HA, HASi and SrHA scaffolds and was identified by comparing the data with the JCPDS files (joint committee for powder diffraction standard). Cytotoxicity of HASi and SrHA scaffolds were evaluated using MTT assay. Cells incubated with extracts from HASi, SrHA and control material HA showed similar metabolic rates of approximately 80%.

B. Fabrication of tissue engineered scaffolds using rat derived MSCs:

Direct contact assay indicated that cells proliferated well surrounding the incubated scaffolds, indicating that the scaffold favored cell proliferation and cytocompatible. Phase contrast images of cytocompatibility studies with SrHA microgranules are represented in figure 2. Staining with DAPI helped to visualize the adhered cells on the cell seeded construct – HA, HASi and SrHA. Osteogenic efficacy assessed in terms of ALP activity indicated that HA, HASi and SrHA scaffolds favoured *in vitro* osteogenesis. TE constructs exhibited highest ALP activity by 21 days of culture, after which there was a decline in the ALP level. Amongst the three scaffolds, cells seeded on SrHA scaffolds exhibited improved ALP production of 13.89±1.5 µmol pNp/ 30 minutes, whereas cells on HA and HASi exhibited ALP levels of 12.44±2.6 and 12.85±2.2 µmol pNp/30 minutes respectively.

C. Development and validation of rat Osteoporotic model:

2D slices (figure 3) generated using micro CT post six and ten months of induction, exhibited a decrease in the trabecular bone volume. In the control animal presence of extensive trabecular bone was observed. Control animals exhibited a Bv/Tv ratio of 0.278±0.007, whereas the induced model exhibited a significantly low Bv/Tv ratio of 0.205±0.009.

D. *In vitro* osteogenic efficacy assessment of the scaffold of choice:

Histological evaluation revealed that the bone defect area was bridged with newly formed woven bone in both HASi and SrHA (bare and cell seeded) groups post 8 weeks of implantation, indicative of the improved osteogenic ability of the modified HA scaffolds. It was interesting to note that the cell seeded scaffolds (cHASi and cSrHA) paved way for more osteoid formation compared to bare scaffolds. Even though cortical bridging was visible in HASi and SrHA implanted groups, but a better resurfacing and *de novo* bone organization at the defect area was evident in cSrHA and SrHA implanted groups, as evident from the histomorphometry analysis (figure 4)

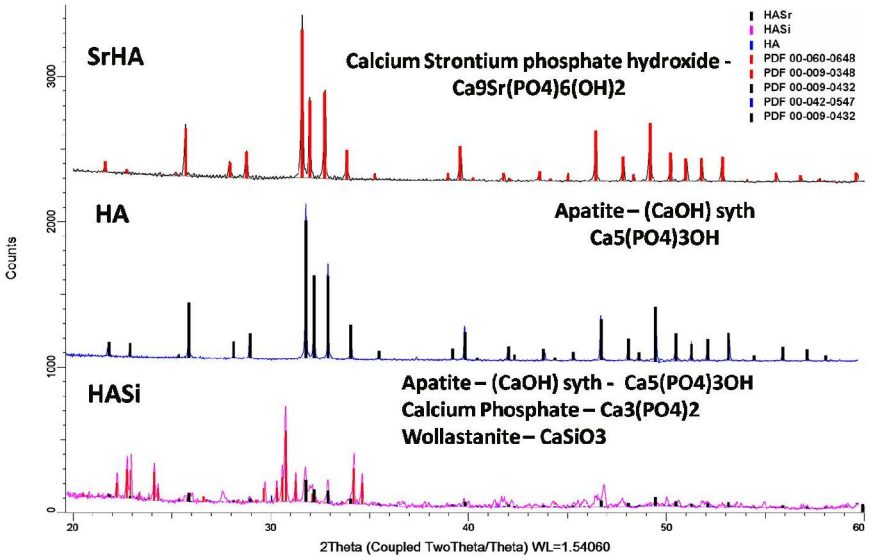

Figure 1 representing the XRD phase identification pattern of HA, HASi and SrHA respectively

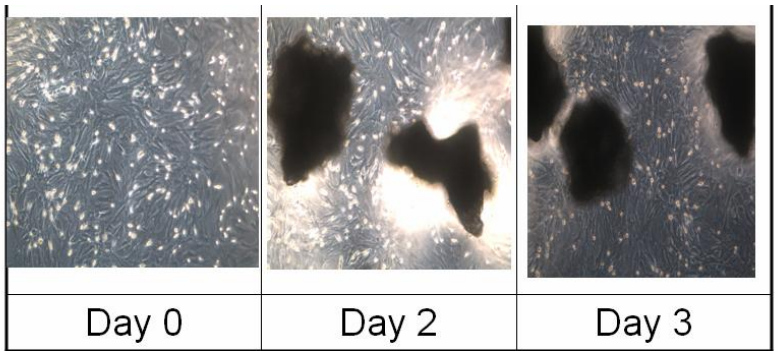

Figure 2.Direct contact assay screening the cytocompatibility of cell ceramic construct with HASr.

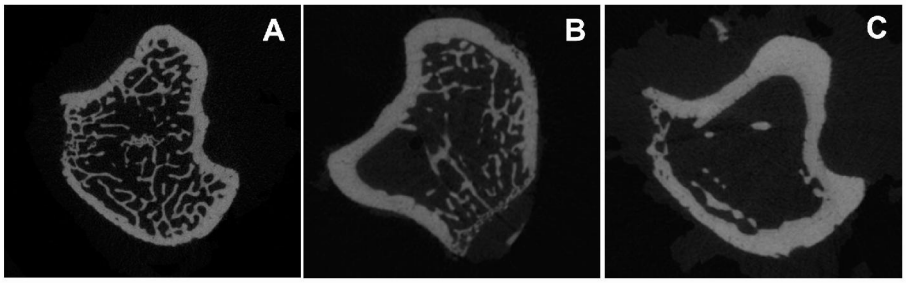

Figure 3 : Evaluation of trabecular bone loss using micro CT depicted the trabecular bone loss in A - control animal, B - 6 months induced and C - 10 months induced rat model.

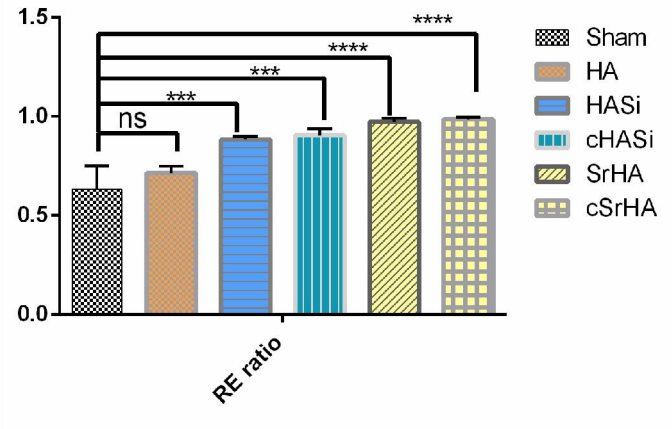

Figure 4 – Quantitative evaluation of regeneration efficiency in osteoporotic model: SrHA and cSrHA implanted group exhibited significantly high RE ratio.

11. PEOPLE'S PARTICIPATION (with emphasis on their involvement in technology generation/ modulation/ adoption/co-operative formation/ self help groups/gender perspective):

- Material synthesis and characterization : Dr. Suresh Babu and Dr. H.K. Varma, Bioceramics Lab, SCTIMST
- Selection of material of choice for osteoporotic application, execution and evaluation of *in vitro* and *in vivo* studies : Dr. Annie John (Project PI) and Mrs. Sunitha Chandran, JRF, TEM lab, SCTIMST
- Animal surgery (rat osteoporotic model development) : Dr. Harikrishan V.S. Scientist D, DLAS, SCTIMST. Mr. Anoop, DLAS, SCTIMST assisted Dr. Harikrishnan in all the animal experiments.

12. PROGRESS INDICATORS FOR MONITORING (Qualitative & Quantitative Analysis - personnel trained, increase in income/productivity, skill upgradation, publications etc as the case may be):

- Sunitha Chandran, Suresh Babu, Harikrishnan V.S., H.K. Varma and Annie John; Osteogenic efficacy of Strontium Hydroxyapatite micro-granules in osteoporotic rat model. (J Biomater Appl 2016 Oct 9;31(4):499-509. Epub 2016 May 9).

13. WORK REMAINING TO BE DONE UNDER THE PROJECT:

A. Osteoporotic model development – Sheep
   1. To develop osteoporotic model by ovariectmoy + Calcium deficient diet.
   2. Evaluation of Sheep osteoporotic model.

B. *In vivo* evaluation of bio-active scaffold in sheep osteoporotic model.
   1. Implantation study
   2. Post implantation evaluation of bone defect healing.

C. To extrapolate data to real clinical situations in women.

14. SPECIAL FEATURES/ HIGHLIGHTS (new technology generation/innovativeness in terms of low cost/ design/environmental friendly etc.)

In vitro and *in vivo* studies in rat osteoporotic model brought out the significance of in house developed Strontium incorporated scaffolds for osteoporotic applications. If similar results may be

obtained in a large osteoporotic animal model like sheep which better mimics human osteoporotic condition, results of the proposed project may find significant clinical application in treating osteoporosis related bone defects. The study has got significant clinical application in terms of the cell source (stem cells) used and the simplicity in the ceramic material preparation. Prime aim of the proposed project is to develop and validate a cost effective in house developed ceramic based scaffold to aid rapid regeneration of the osteoporotic bone defects.

15. EQUIPMENTS SANCTIONED AND PROCURED:

Cell culture CO2 incubator and pare parts of perfusion bioreactor sanctioned in the project and has been procured.

16. STAFF SANCTIONED:
STAFF IN POSITION:

Junior Research Fellow : Mrs. Sunitha Chandran - October 2015 to October 2016. (Relieved from project)
Animal Handler – Mr. Anoop – October 2015 to October 2016. (Relieved from project)

17. CONSTRAINTS IF ANY:

Second year fund was not allotted and hence project staffs have to be relieved in October, 2016. Out of Rs.12,25,516/-_remaining unutilized, Rs. 7,43,551/- is committed for incubator, as purchase orders were processed before March, but the incubator was delivered only by October 2016.

DATE: 16-01-2017 **SIGNATURE OF PI:**

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Dr. P.R. HARIKRISHNA VARMA
Head, BMT Wing