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PROJECT COMPLETION REPORT

1. **Project Number** : P8222

2. **Title of the Project** : Bioceramic cages with axially aligned pores as a substitute for tricortical bone graft

3. **Funding Agency Name** : DST supported TRC scheme @ SCTIMST

4. **Project Reference Number provided by the Funding Agency**: TRC/8222

5. **Principal Investigators (Name & Address) :**
   i) Dr. Manoj Komath, SCTIMST Tvm
   ii) Dr. Naresh Kasoju, SCTIMST Tvm

6. **Co-Investigators (Name & Address):**
   i) Dr. Harikrishna Varma PR, SCTIMST Tvm
   ii) Dr. Anil Kumar PR, SCTIMST Tvm
   iii) Dr. Anoop Pillai, Orthopaedic Consultant, SUT, Trivandrum

7. **Implementing Institution** : SCTIMST Trivandrum

8. **Collaborating Institutions** : N/A

9. **Date of Commencement** : 09 Oct 2019

10. **Duration** : 1.5 Years

11. **Date of Completion** : 08 Apr 2020

12. **Objectives as approved:**
    i) To design and develop a ceramic cage which is axially load bearing with aligned pores distributed around the central part.
    ii) To investigate various mechanical and biological parameters of the ceramic cage for meeting essential clinical requirements.
    iii) To design and develop a bioreactor for dynamic culture studies and evaluate response of mesenchymal stem cells seeded on ceramic cages.

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

14. Field/Experimental work giving full details of summary of methods adopted, data collected supported by necessary tables, charts, diagrams and photographs : *Kindly refer to the details presented in S. No. 16.*

15. Detailed anaylsis of results : *Kindly refer to the details presented in S. No. 16.*

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

Title: Bioceramic cages with axially aligned pores as a substitute for tricortical bone graft

Introduction and Rationale:

The current procedure for autografting involves a second surgery. A specific shaped and structured graft piece (tricortical autologous bone) harvested from patients' iliac crest solves the problem mostly, but at the cost of harvest site morbidity. Synthetic porous bioceramic grafts are a good option for avoiding the second surgery. However, bioceramic grafts did not prove an alternative because (i) they lack osteoinductive factors (ii) the random porosity structure will not lead to mechanotransduction and (iii) low machinablilty, which makes it difficult to shape according to surgical needs.

In the present work, a cylindrical bioceramic cage structure with axially aligned pores will be designed through a special slipcasting technique. This will be a silicate glass-ceramic structure which will be machinable and cut in transverse direction easily. Because of the architecture, the graft will be loadbearing in the axial direction. In the in vivo grafting procedure, osteoinductive factors could be imbibed in the graft by infusing autologous blood or bone marrow aspirate.

Objectives:

i) To design and develop a ceramic cage which is axially load bearing with aligned pores distributed around the central part.
ii) To investigate various physico-chemical and biological parameters of the ceramic cage for meeting essential clinical requirements.
iii) To design and develop a bioreactor for dynamic culture studies and evaluate response of mesenchymal stem cells seeded on ceramic cages.

Methodology:

Objective 1. To design and develop a ceramic cage which is axially load bearing with aligned pores distributed around the central part

Hydroxyapatite (HA) powder for the graft was synthesized by an inhouse developed method reported elsewhere. The samples of HA ceramics were made using the slip casting method wherein slips were prepared with the HA powder homogeneously distributed in a solution containing dispersant (to suspend the particles) and binder (to keep the shape of the green body). The optimal slip was made from the solution with 4 w/v polyvinyl alcohol as a binder and 0.4 w/v sodium salt of polyacrylic acid as a dispersant in distilled water, in which HA was mixed vigorously using a stirrer up to 50 vol%. These were poured into molds with higher size than the required sample dimensions, to compensate for sample shrinkage while sintering.

Objective 2. To investigate various physico-chemical and biological parameters of the ceramic cage for meeting essential clinical requirements.

The surface micro-morphology of the HA bioceramic samples was examined under a scanning electron microscope (SEM, model Hitachi S-2400). The phase purity and crystallinity of the hydroxyapatite ceramic were investigated using X-ray powder diffraction (XRD). The spectrum obtained was compared with the International Centre for Diffraction Data (ICDD) through inbuilt software to identify the phases in the material. The phase information was appended with the functional group analysis using Fourier Transform Infrared Spectroscopy (FTIR). Subsequently, HA samples with aligned through-pores were prepared and explored in vitro, with a focus on how the pores host the cells inside and to what level the cells maintain their activity. Human osteoblast-like cells (HOS) were used, at different seeding and culturing approaches.

Objective 3. To design and develop a bioreactor for dynamic culture studies and evaluate response of mesenchymal stem cells seeded on ceramic cages

In the current study, we hypothesize that aligned through pores in the graft will lead to a faster healing by homing the local cells inside and provide a better environment for new bone formation through graft structure. The investigation was done using aligned porous HA scaffolds seeded with human Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs). For providing dynamic culture conditions, we have developed a novel multiwell format bioreactor setup. The cell adhesion was studied by microscopy, cell proliferation was evaluated by Alamar blue assay and osteogenic differentiation was confirmed by biochemical and molecular assays. (Stem cell study was approved vide reference SCT/ICSCR/56/December2019).

Results:

In this study, the fabrication and effectiveness of cage-like HA ceramic design with end-to-end open pores has been investigated, with the help of in vitro cell culture methods. Following are key observations:

As for the pore structure of the scaffold, in the current study, HA based bioceramic cages having aligned channeled were designed and developed by slip-casting and sintering, wherein through-pores of diameter about 250 μm were obtained. The material characteristics were analyzed by SEM, EDS, XRD and FTIR and the results were found to be an implantable bioceramic for bone defects.

The current study explored a strategy for enhanced cell seeding and culture within these aligned porous cages. Our results with HOS cells indicated that a cell seeding approach of placing a concentrated drop of cell suspension directly on top of the cage end portion gives the best cell adhesion and proliferation.

A simple and versatile dynamic culture system has been designed and developed comprising a multi-well plate with specialized inserts that can hold cell-laden cages, placed on a rocker platform inside a CO2 incubator. This way, the cell infiltration was found to be much deeper and the cell proliferation after 1 week of culture was significantly higher in the aligned porous cages.

The samples in the differentiation medium showed distinct calcium crystals and composition in SEM/EDAX analysis, indicating the successful differentiation of hWJ-MSCs and subsequent mineralization. This was further confirmed by reverse transcription-qPCR analysis, wherein stemness (CD73 and CD90) and osteogenic (RUNX2 and ALP) markers were found positive.

Overall, MSCs, axially aligned porous bioceramic scaffolds, and a multiwell plate format bioreactor-based bone tissue engineering approach enabled the successful fabrication of biomimetic bone tissue grafts for potential application in clinical setups.

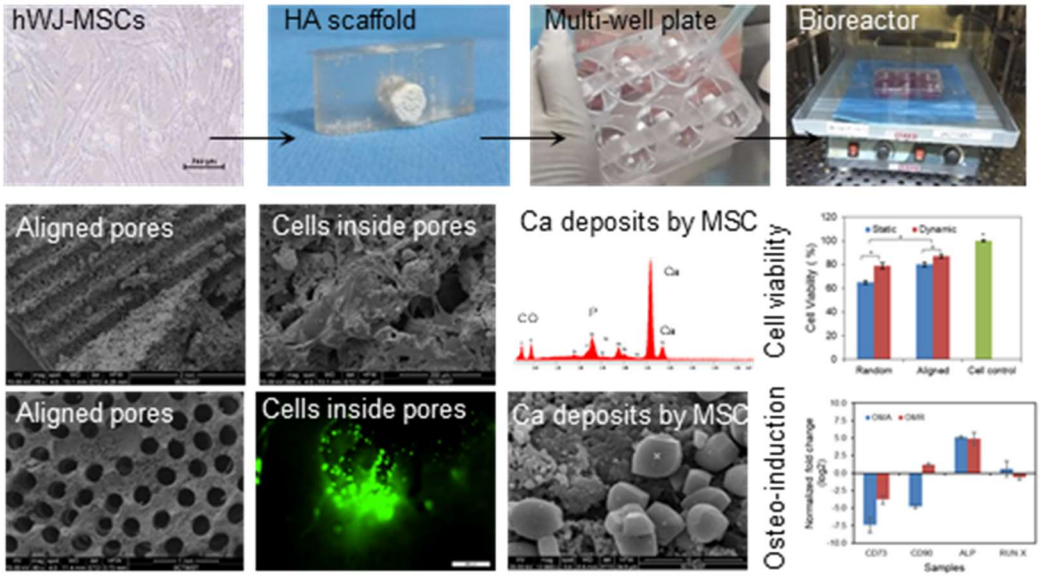

Figure. Representative data showing axially porous bioceramic cages and the in vitro performance with human mesenchymal stem cells in a multi-well format bioreactor.

Translational potential:
The project involves the development of cylindrical bioceramic cage structures with axially aligned through-pores and testing their in vitro biological response. It is proposed that the new bioceramic cage structure, infused with autologous blood or bone marrow aspirate, may obviate the need for tricortical graft which is harvested from the patient through a second surgery. The osteoinductive factors in the cage structure could trigger osteogenic differentiation of resident stem cells. To test this hypothesis, an in vitro study was designed wherein the human mesenchymal stem cells were seeded onto the bioceramic cages. A specially designed multi-well format bioreactor setup was used in the study to aid high-throughput in vitro culture. It was observed that the cells seeded into the cages successfully differentiated into osteogenic lineage upon induction. Similar positive results were obtained when the culture was done in dynamic conditions, too. The project outcome is an indicator that the bioceramic cages with autologous osteogenic cues will serve as a substitute for tricortical autologous graft.

17. **Contributions made towards increasing the state of knowledge in the subject :**
The data collectively endorse that the axially aligned porous cages combined with a multi-well format based bioreactor assisted dynamic culture approach described in this study could offer a potential solution in developing in vitro engineered bone tissue grafts that could be explored in intervertebral fusion and long bone fracture non-union where faster healing and quicker site strengthening are needed.

18. **Conclusions summarising the achievements and indication of scope for future work :** *Kindly refer to the details presented in S. No. 16.*

19. Science and Technology benefits accrued :

a. List of publications with complete details : 02

(i) Gayathry G†, Athira RK†, Anju MS, Anil Kumar PR, Harikrishna Varma PR, Kasoju N*, Manoj Komath. Mesenchymal stem cell culture in aligned porous hydroxyapatite scaffolds using a multi-well plate bioreactor for bone tissue engineering. **MedComm – Future Medicine** 2022;1: e17.

(ii) Athira RK†, Gayathry G†, Anil Kumar PR, Harikrishna Varma PR, Kasoju N*, Komath M*. Hydroxyapatite cages with aligned pores for bone grafting – seeding of human osteoblast-like cells in vitro and their response in dynamic culture mode. **Ceramics International** 2021; 47: 30051-30060.

Conference presentations: 02

i) Athira RK, Gayathry G, Komath M, Kasoju N*. Bioceramic scaffolds with aligned pore distribution seeded with human mesenchymal stem cells for bone grafting – an in vitro study. National Conference on Recent Trends in Materials Science and Technology. Organized by Dept. of Chemistry, Indian Institute of Space Science and Technology, Thiruvananthapuram (29-31 Dec 2021).

ii) Athira RK, Gayathry G, Anil Kumar PR, Komath M, Kasoju N*. Effects of pore structure and cell culture approach on in vitro cellular response to bioceramic samples. International Conference on Biomedical Materials Innovations 2020. Organized by Bharathiar University, Coimbatore in association with SBAOI and STERMI (06-09 Dec 2020).

b. Manpower trained on the project :

i.	**Research Scientists or Research Fellows**	**:**	01
ii.	**No. of PhD's produced**	**:**	01 (ongoing)
iii.	**Other Technical Personnel trained**	**:**	N/A

c. Patents taken, if any **:** 02

i) Kasoju N, Komath M, Anil Kumar PR, Harikrishna Varma PR, Athira RK. Multi-well plate with inserts for cell culture and biomedical applications. No. 202141005890, Dt. 11/02/2021 (patent)

ii) Kasoju N, Komath M, Anil Kumar PR, Ramesh Babu V, Athira RK. Multi-well cell culture plate with inserts. No. 330128-001, Dt. 17/06/2020. (design registration)

d. Products developed, if any **:** N/A

20 Abstract: (In 300 words for possible publication)

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The project involves the development of cylindrical bioceramic cage structures with axially aligned through-pores and testing their in vitro biological response. The cages were made through slip-casting technique using optimised slurries containing hydroxyapatite or silicate glass-ceramic powders along with binders and dispersants. Linear pore-forming fibers were placed along a specific direction in the cast, uniformly spaced, so that they will sublime while sintering in the furnace. It creates a scaffold structure similar to the cortico-cancellous architecture of a tricortical bone graft. It is proposed that the new bioceramic cage structure, infused

with autologous blood or bone marrow aspirate, may obviate the need for tricortical graft which is harvested from the patient through a second surgery. The osteoinductive factors in the cage structure could trigger osteogenic differentiation of resident stem cells. To test this hypothesis, an in vitro study was designed wherein the human mesenchymal stem cells were seeded onto the bioceramic cages. A specially designed multi-well format bioreactor setup was used in the study to aid high-throughput in vitro culture. It was observed that the cells seeded into the cages successfully differentiated into osteogenic lineage upon induction. Similar positive results were obtained when the culture was done in dynamic conditions, too. The project outcome is an indicator that the bioceramic cages with autologous osteogenic cues will serve as a substitute for tricortical autologous graft.

21 Procurement/Usage of Equipment: N/A

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a. Details of Equipment:

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

b. Suggestions for disposal of equipment(s): N/A

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