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TITLE: CONTROLLED RELEASE OF INACTIVATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE: A SINGLE VACCINATION APPROACH FOR LONG-TERM IMMUNITY

PRINCIPAL INVESTIGATOR: DR. JOSEPH J. MATTAPALLIL,

CONTRACTING ORGANIZATION: THE HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, INC.

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DR. JOSEPH J. MATTAPALLIL

E-Mail: joseph.mattapallil@usuhs.edu

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HENRY M JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, INC.
6720A ROCKLEDGE DRIVE
BETHESDA, MARYLAND 20817

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14. ABSTRACT

Requirement of multi dose regimen of vaccines presents a barrier to protect from vaccine preventable disease. Problem is amplified in case of emerging infectious viruses where either there is a lack of live attenuated strain of virus to immunize and for inactivated vaccine not much time is at hand to perform multiple immunizations. Chikungunya virus (CHIKV) has caused large outbreak in tropical countries across the globe. There is no FDA approved vaccine against CHIKV. In this study gamma-radiation inactivated CHIK181/25 is encapsulated in lipid nanoparticles (HALNP) which will be embedded in the polyelectrolyte multilayer (PEM) films for temporal release in vivo. This platform will provide a single-shot vaccine of inactivated-CHIKV181/25 for intermittent release of antigen mimicking primary and booster regimen of multi-dose vaccination. CHIKV181/25 was inactivated by gamma radiation using a unique approach of protecting viral proteins while allowing for homogenous inactivation of the virus. Protection of epitopes on inactivated-CHIKV181/25 was determined and encapsulation of inactivated-CHIKV181/25 in HALNP was performed. Mice were immunized with HALNP encapsulated inactivated-CHIKV181/25 and anti-CHIKV antibody titers were evaluated to establish antigen preservation post encapsulation. Experiments are now planned to test the long-term immunity following immunization with HALNP-encapsulated inactivated CHIKV181/25 embedded in the PEM lattice. This study addresses an FY17 PRMRP topic "Vaccine Development for Infectious Disease" and Area of Encouragement under "Development of vaccines to prevent U.S. Service members from becoming ill from endemic disease exposure during operational deployments including arthropod-borne diseases such as chikungunya virus".

15. SUBJECT TERMS
Chikungunya virus, encapsulation, hyaluronic acid coated lipid nanoparticles, polyelectrolyte multilayer films, slow release, long term immunity.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   Chikungunya virus (CHKV) is a mosquito borne arthritogenic alphavirus that causes acute rheumatic fever, arthritis, arthralgia, and muscular pain. Though controlled early, symptoms usually persist for 2-3 years after onset in in a large fraction of infected individuals leading to chronic arthritis like symptoms. Major CHIKV outbreaks have been reported in Asia, Africa and more recently in Latin America that poses a significant risk to US Military personnel who are deployed to these areas. CHIKV is a BSL3 pathogen and listed as a Category B pathogen by the U.S. Department of Homeland Security and the Centers for Disease Control and Prevention and are easy to disseminate and require enhanced disease surveillance. There are currently no licensed vaccines or effective therapies against CHKV infection. As such there is an urgent need to develop a vaccine that can give long-term protection from infection. Though numerous vaccine candidates are being tested in the field, most of these vaccines need multiple doses to induce protective immunity. A multi-dose vaccine poses a number of compliance challenges such as vaccine hesitancy, resource and time constraints related to mass immunizations in the event of an outbreak, safety profiles etc. A whole inactivated vaccine that can be administered as a single dose vaccine yet being capable of inducing long-term protection would have a significant impact during an outbreak. We propose to advance such an approach by packaging whole inactivated CHIKV in liposomal nanostructures and embedding them in polyelectrolyte multi layer films (PEM).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Chikungunya virus, encapsulation, hyaluronic acid coated lipid nanoparticles, polyelectrolyte multilayer films, slow release, land long-term immunity.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   1. Encapsulate MDP-iCHIKV in PEM film lattice for programmed release.
   2. Test the long-term immunity against CHIKV after immunization with PEM encapsulated MDP-iCHIKV.

   What was accomplished under these goals?
   For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project

progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Stock of CHIKV 181/25 virus stock was amplified and purified for generating the MDP-iCHIKV for PEM encapsulation and immunization experiments.
2) Virus stock was inactivated by gamma radiation (20kGy) in the presence of MDP complex (Mn2+:decapeptite:PO4:: 1:3: 25mM) using an in house Co60 irradiator to generate MDP-iCHIKV. Inactivation was confirmed using in vitro assays and the preservation of viral epitopes was determined by Western blot.
3) MDP-iCHIKV was successfully incorporated into HALNP nanoparticles for use in immunization studies. Timed release of MDP-iCHIKV was confirmed used ELISA assays.
4) Proof-of concept studies in mice were initiated to demonstrate immunogenicity by measuring CHIKV specific IgM and IgG in the serum of immunized animals. HALNP encapsulated MDP-iCHIKV induced IgG titres similar to that of MDP-ICHKV alone.
5) Timed-release encapsulation of MDP-iCHIKV in HALNP-PEM was carried out and tested in vitro. After confirming release, mice were immunized using different combinations of MDP-iCHIKV-PEM to test for in vivo immunogenicity. The following groups were used:

   1. **Early release (ER)**: PLGA-$(PLL\text{-}SPS)_{4.5}$-HALNP
   2. **Delayed release 1 (DR-1)**: PLGA-$(PLL\text{-}SPS)_{4.5}$-HALNP-$(PLL\text{-}SPS)_{4.5}$
   3**. Delayed release 2 (DR-2)**: PLGA-$(PLL\text{-}SPS)_{4.5}$-HALNP-$(PLL\text{-}SPS)_{10.5}$
   4. **Early+Delayed release 1 (ER-DR1)**: PLGA-$(PLL\text{-}SPS)_{4.5}$-HALNP-$(PLL\text{-}SPS)_{4.5}$-HALNP
   5. **Early+Delayed release 2 (ER-DR2)**: PLGA-$(PLL\text{-}SPS)_{4.5}$-HALNP-$(PLL\text{-}SPS)_{10.5}$-HALNP

   Our preliminary results demonstrated that Group ER-DR1 and ER-DR-2 had higher IgG titres compared to other groups. We propose to explore the use of one of these approaches in subsequent animal studies.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Covid-19 related disruptions continued to constrain opportunities for training.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We are in the process of confirming our preliminary findings from the mouse immunization studies following which we intend to publish these in a peer reviewed journal and also present them at the 2022 MSHRS meeting.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period we propose to undertake studies using mice with the intent of comparing immune responses over a long duration and include both humoral and cellular immune responses. Given the low volume of blood that is obtainable from mice bled sequentially, we may combine approaches such as Cite-Seq that yields data on the different subsets and related gene expression changes within these subsets. By comparing mice that are immunized with two doses of MDP-iCHIKV to single dose MDP-iCHIKV-PEM and control mice we will be able to determine if MDP-iCHIKV-PEM induces an immune profile that si either similar or better than MDP-iCHIKV alone.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Our preliminary findings suggest that a single dose vaccine could induce potent immune responses that may translate to better protection following infection with CHIKV. The proposed vaccine strategy could obviate the need to multiple vaccinations to induce protective immunity.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The single dose encapsulation approach has significant potential to impact other viral vaccine candidates against numerous emerging infectious diseases that are potential threats to US Military personnel, and civilian population in general.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *ortadoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

No major changes are planned except that we may incorporate use of Cite-Seq to examine gene expression changes in different subsets of cells over a period of time to assess if MDP-iCHIKV-PEM induces better quality immune responses as compared to MDP-iCHIKV alone or control groups. The approach is similar to flow cytometry but yields significantly more information that could inform potential correlates of protection.

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Covid-19 related disruptions continue to constrain the execution of the project. However, we anticipate that these constrains will ease during the fall of 2021 and beyond that will allow us initiate the studies we had outlined. Though we anticipate completing the animal studies by January of 2022, the different assays (flow cytometry, neutralizing and binding antibody assays, gene expression analysis etc) will need significantly more time for us to complete.

Dr. Kidambi's laboratory was shut down due to Covid-19 from March of 2021 to July of 2021 as University of Nebraska shut down its activities that significantly impacted animal studies at USU. Since reopening in July, Dr. Kidambi's laboratory has been functioning with limited access to personnel with only one individual allowed to work in the laboratory at a time that has delayed the onset of the animal immunization studies.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No major changes in expenditures are anticipated. I inherited this grant from a previous PI who left USU. As such there were expenditures related to this transition such as the purchase of various reagents and supplies, mice anesthesia unit for longitudinal collection of samples from immunized mice, euthanasia equipment etc. The limited amount of funds that were available in the grant after it was transferred to me was a constraint on hiring a fully trained technician with experience in virology and immunology assays.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.
  - A novel inactivated-chikungunya virus vaccine, MHSRS, September 19-22, 2019, Kissimmie, FL.
  - rradiated Chikungunya Vaccine and Host-directed Anti-viral Therapeutic, Gerogia State University, Friday, April 26, 2019.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  None.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  None.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  None.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  None.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  None.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  None.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Joseph J Mattapallil
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2

Contribution to Project:	Design and execution of animal studies, virology and immunology assays.
Funding Support:	Dr. Mattapallil is a federal employee. As such no funding support was provided.

Name:	Srivatsan Kidambi
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.25 summer months
Contribution to Project:	Design and encapsulation of MDP-iCHIKV into HALNP-PEM nanoparticles, testing timed release of antigen.
Funding Support:	None.

Name:	Bianca Galasso
Project Role:	Laboratory Technician 1
Researcher Identifier	N/A
Nearest person month worked:	0.35
Contribution to Project:	Worked under close supervision of the PI in conducting experiments proposed and assist in data analysis
Funding Support:	This award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial

or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name: ***University of Nebraska***
*Location of Organization: (if foreign location list country)****: Nebraska, USA***
Partner's contribution to the project (identify one or more)

- *Financial support;* ***None***
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);* ***None***
- *Facilities (e.g., project staff use the partner's facilities for project activities);* ***Dr. Kidambi's laboratory is used for preparing HALNP-PEM-MDP-iCHIKV.***
- *Collaboration (e.g., partner's staff work with project staff on the project);* ***Dr. Kidambi and his staff prepare the HALNP-PEM-MDP-iCHIKV for immunizing mice.***
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.