Mécanismes et mécanique en systèmes multicellulaires tridimensionnels

Mechanisms and mechanics in 3D multicellular systems

Mecacell3D

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PREAMBLE

Challenges and issues in health and biology are constantly changing and opening new directions for innovation. In this context and in line with its missions, which consist of accelerating progress in knowledge, supporting integrated and multidisciplinary research, and ensuring a continuum between fundamental and clinical research, Inserm is putting in place cross-cutting scientific programs with the following objectives:

- to build scientific communities in specific high-priority fields and bring forth national **interdisciplinary consortia** that will build on the skills and expertise of Inserm teams;
- to make French biomedical research a leading player in these fields by accelerating knowledge acquisition, transfer, and value-creation.

These unifying programs aim to create a new dynamic in innovative fields by developing complementary skills to explore research niches that have so far been little studied. Funding will only be provided to collaborative projects that spread across a group of activities. These programs are open to academic and industrial partnerships with a flexible approach: such partnerships may span either the whole program or only one or more of the program tasks.
Scientific questioning at the frontier of biological knowledge, new technological opportunities, pooling the strengths of Inserm teams working in the field of the cross-cutting program, and potential added benefits in terms of societal value creation constitute the determining elements for implementing these programs.

GENERAL OBJECTIVES OF THE Mecacell3D PROGRAM

Although the study of cells grown in 2 dimensions has yielded invaluable data, these models have inherent limitations. There has thus been lately a worldwide **effort to generate 3D cell models that better recapitulate the *in vivo* situation whether normal or pathological**. This in turn, induces the **need for an adaptation of cell biology tools to explore these models adequately**. At the same time, most 3D models remain of limited complexity, lacking, for instance, non-epithelial cell types, blood vessels, and/or innervation.

This program aims at developing and/or adapting quantitative methods to characterize cell functions in three-dimensional cell assemblies, considering their individual or collective mechanical properties. This objective should be carried out by using highly relevant and reproducible models and by permitting quantitative analyses.

As detailed later (see page 8), teams may apply either alone or as collaborative "mini-consortia". Ultimately, all selected teams will organize themselves within a single consortium under the responsibility of a scientific coordinator.

SPECIFIC OBJECTIVES OF THE CROSS-CUTTING PROGRAM

The following should be viewed as a general framework illustrated with a few examples. It is by no means an exhaustive list of potential objectives.

TASK 1. Development and validation of complex 3D multicellular MODELS

Context and objectives

Cellular models are essential in biological research to help with deciphering physiological and pathophysiological mechanisms. Since the 50's, 2D cell culture has been extensively used, and

a growing number of different cell lines have been made available. However, those are too simplistic models, not well representative of the actual cell environment of tissues in the body. Strategies have been developed to generate 3D models in order to overcome 2D models limitations, while recapitulating at best the *in vivo* situation, Therefore, 3D structures namely spheroids, organoids and tumoroids have thrived over the past two decades. Nevertheless, 3D cultures still exhibit several shortcomings: lack of reproducibility; lack of specificity concerning cell-type(s) composition; uncontrolled size; shape heterogeneity; absence of proper vascular, immune and innervation components; and incomplete functionality. Organoid culture is also costly and time consuming, with variable rates of efficiency in generation and maintenance. Therefore, strictly standardized and robust sample preparation is a crucial development.

Many complex *in vitro* models are currently used, but they are often composed of only one or a few cell types present in an oversimplified or artificial environment. For instance, most models lack vasculature, which is necessary for proper gas exchange and widespread access to soluble factors. In addition, many tissues are subjected to mechanical forces, such as fluid pressure, shear stress, compression or tensile stress, stretching, spatial confinement, all of which modulating their function though yet poorly recapitulated *in vitro*. Further, stromal cells or extracellular matrices with different rheological properties can also affect cell behavior and orient cell fate as will the different immune cells present in the tissues.

Thus, the objective is to develop/improve and validate suitable 3D models that better recapitulate specific aspects of tissue homeostasis or disease, allowing detailed characterization of cell biology processes in those complex environments. Because such models may not fully recapitulate the *in vivo* situation, they will have to be accompanied by cross-validation in an *in vivo* model.

Scientific questions and obstacles

The major obstacle in achieving this objective is to find a solution for combining complexity - at a level sufficient to fulfill physiological relevance, with robustness - required for reproducible quantification of cell biological processes and/or screening of inhibitory/activating molecules through high-resolution imaging and single cell /spatial omics.

The consortium should focus on a limited number of examples - modeling one or two tissue types/organs. The model should consist of embryos, organoids, or non-organoid assemblies of several cell types embedded in complex extracellular matrix scaffolds and allow for controlled environment modifications (including microfluidic approaches to mimic blood vessels, for instance). The model should also be built to achieve reproducibility (for example, adopting bio-impression and micro-patterning techniques). Finally, the system should be validated to faithfully recapitulate certain pathophysiological features of specified tissue/organ (by comparing major aspects with tissues/organs *in vivo* or *ex vivo*).

Here are examples of specific challenges that could be addressed:

- Standardized and robust sample preparation
- Adapt chemical and biomechanical environment of cells:
  - culture media that support growth and proper function of different cell types

- molecular composition and mechanical properties of scaffolds
- design of new, well-defined extracellular matrices for 3D culture
- controlled media and gas exchange
- controlled or tunable rheology

–Set-up and validation of robust methods for overcoming physico-chemical constraints for:
- bioavailability and biodistribution of exogenous molecules within the 3D structures
- deep and/or high-resolution imaging despite inherent light penetration limitations

–Validate *the model in vivo* or *ex vivo* using data from the literature or direct observation

Deliverables

Develop a physiologically relevant and robust *in vitro* model system that recapitulates defined aspects of tissue/organ homeostasis or disease and allows precise measurements and quantification of processes at the cell and sub-cellular levels.

TASK 2. Developing quantitative molecular and cellular-based ASSAYS in 3D cellular models

Context and objectives

Beyond improving 3D cultures engineering and searching for a patho/physio-mimetic complexity, it is important to develop, adapt, or improve cell biology methods. Probing cell heterogeneity and cell crosstalk while retaining spatial information in the 3D cultures is a challenge of primary importance. Procedures for standardized and robust sample preparation are most desired.

Thus, the objective is to develop molecular and cellular assays for 3D models and demonstrate sensitivity, reproducibility, and robustness by comparison to the 2D setting.

Scientific questions and obstacles

A major obstacle resides in adapting microscopy techniques to resolve the time/space/resolution conundrum in 3D assemblies. Another one is to reach single cell analysis while keeping the spatial information of the different cells.

The consortium should focus on a limited number of examples: a limited number of 3D assemblies of cells and a limited number of assays.

A few examples are provided below.

Optimization of technologies probing cell mechanics

Several techniques allow to probe cell and tissue mechanical forces, but their applicability is mainly restricted to single cells and monolayers *in vivo* and *in vitro*. These include the use of genetically encoded fluorescence resonance energy transfer sensors, Traction Force Microscopy, laser ablation, or optical force inference.

Investigating intracellular/ intercellular dynamics and cell signaling

It is necessary to transfer the recent advances of high-resolution optical imaging to the 3D context. Unbiased quantitative analysis of macroscopic biological samples demands fast imaging systems capable of maintaining high resolution across large volumes. The same is true

for single-cell omics and spatial omics that allow maintaining the inter-cellular cross-talks missing at the single-cell level. A particular focus on intracellular trafficking (endocytosis, exocytosis, mitochondria, and other compartment dynamics), cell signaling (from the cell surface to the cytoplasm to intracellular membranes and the nucleus and vice-versa), and cellular crosstalk is expected.

Solving accessibility issues in 3D
Methods using physical probes such as tether pulling, or atomic force microscopy are not yet adapted to study the mechanical properties of spheroids or organoids. Applying drugs or tracers in a 3D context is also challenging as it is difficult to estimate if they penetrate properly and evenly target all cells in the 3D assembly.

Examples of deliverables

- Cleared 3D samples for 3D imaging with depth resolution and subcellular resolution
- Development of volumetric fluorescence imaging
- Powerful multiscale imaging tool for studying 3D structures of macromolecular assemblies
- Advanced 3D imaging modalities, including label-free molecular imaging
- Optogenetic application in 3D structures
- Spatial Omics toward single cell resolution
- Spatio-temporal signaling in 3D structures

TASK 3. Data ANALYSIS and theoretical MODELING of 3D cellular assemblies

Context and objectives

The methodologies and techniques described in Task 2 need to be quantitative and request refined analysis. These analyses are required to fully exploit the results of challenging experiments on sophisticated *in vitro* cellular systems, and they should thus be an integral part of the proposal.

The objective is to build, develop and/or adapt the methods for a rigorous and quantitative analysis of the data generated by the assays developed in WP2.

Scientific questions and obstacles

It is suggested that analytical methods fall within 3 broad groups: algorithm optimization, bioinformatics, simulations. This is by no means an exhaustive list, and other types of analysis will be considered if they tackle the challenges that arise when studying multicellular 3D instead of 2D contexts. The main obstacle lies in the potential mass of data generated when considering, for instance, high resolution 4D imaging of multiple probes and single cell multi-omics.

The consortium should focus on a limited number of analytical methods, preferably on those adapted to the assays developed in WP2.

Below are given a few examples of the challenges that could be addressed within this Task.

Algorithm optimization

In general, 3D experiments will be much more demanding in terms of data volume and time for analysis. There is a need to optimize algorithms that should run more quickly and hopefully reduce the time for off-line analysis. These could be applied, for instance, to the following methods:

- Traction Force Microscopy
- Optical force inference.
- Holographic-based techniques.
- Label-free imaging techniques where notably AI could be advantageously used
- Organelle segmentation and morphometrics

Bioinformatics

- Analysis of single cell transcriptomics and proteomics or regionalized microdissection-based omics.
- Combination of several imaging modalities or imaging with mechanical stress application, drug penetration, etc

Simulations

- Digital twins. To face the huge parameter space generated within 3D co-cultures, generation of *in silico* models is needed.

Examples of deliverables

- Multimodal imaging techniques for 3D live imaging with subcellular and, hopefully, molecular resolution
- Digital twins
- Simplified, online, rapid image analysis based on optimized algorithms or AI for optical force inference and label-free imaging

3 OPERATION OF THE CROSS-CUTTING PROGRAM.

Governance and organization

The cross-cutting program: based on the formation of a scientific consortium, organized around scientific working axes, each composed of a number of scientific teams that may vary depending on the objectives. This consortium will be led by a scientific coordinator and guided by the heads of each working axis.

The scientific expert committee: responsible for selecting the letters of intent, producing recommendations for the directions of the cross-cutting program, advising on alignments across the teams to form working axes, and approving the final scientific project. It is composed of international outside experts and directors of the relevant Inserm thematic institutes.

The scientific monitoring committee: responsible for monitoring the progress of the scientific project. It is composed of the consortium scientific coordinator and the scientific leads from each working axis.

The program management committee: responsible for managing the running of the program, including the budget, and approving proposals from the scientific monitoring committee for activities relating to the implementation of the overall program strategy. It is composed of the legal representative of the coordinating institution and the director of the thematic institute relevant to the theme of the program, in this case, the Cell Biology, Development, and Evolution Institute.

Program set-up

Preparation of the cross-cutting program

A working group composed of field experts[1] has compiled a list of the relevant scientific issues to bring together complementary skills. Their discussions have culminated in the proposed program definition.

Consortium set-up

The consortium is organized around tasks. Participation in the different tasks will occur in two stages: an initial selection of candidates by the scientific expert committee based on letters of intent (see Evaluation criteria p. 9), followed by a phase of co-construction of working axes (see p. 9).

Submission of the letter of intent

Any proposal should address **at least 2 of the 3 tasks** described in the program. The letter of intent should specify which program tasks will be addressed by the team and describe how the researchers or team's skills and expertise may contribute to overcoming one or more of the conceptual and/or technological obstacles identified as high-priority scientific components of the cross-cutting program. The proposals can originate from either:

1- A single researcher or a research team able to address at least 2 out of 3 tasks.
2- A group of 2 to 3 teams (mini-consortium) addressing 2 to 3 tasks.

[1] Composition of the expert group: Corinne Albiges-Rizo (Grenoble), Isabelle Fournier-Salzet (Lille), Jacky Goetz (Strasbourg), Christophe Lamaze (Paris), Guillaume Montagnac (Villejuif), Pierre Nassoy (Bordeaux), Marie-Hélène Verlhac (Paris), Danijela Vignjevic (Paris)

Co-construction of working axes

The international scientific expert committee will select projects/teams based on the evaluation criteria described below. Importantly, if a project is submitted by a "mini-consortium", the expert committee may decide to retain only some but not all the constituent teams.

Following the selection of letters of intent, the coordinating institution (Inserm), based on the proposals and recommendations of the scientific expert committee, will invite the selected candidates to group themselves by working axes and contribute to drafting a scientific project. It is expected that each axis should encompass **Model** development, setting-up of **Assays**, and data **Analysis** and **Modeling**. This project will be presented to the steering committee and the scientific expert committee as a discussion seminar.

Following this seminar and taking on board the committee recommendations, the consortium scientific coordinator will file a final scientific project with the coordinating institution (Inserm) that details the contribution of each team, the objectives, and the expected added benefits. Following the formation of the program, a 3-year funding plan will be detailed, and external funding sources identified.

Follow-up of the consortium

The steering committee will organize an annual general meeting that brings together, in addition to its committee, the scientific expert committee and the scientists involved in the consortium. During this meeting, participants will present and discuss the progress of the cross-cutting program, the next stages to tackle, and, if necessary, propose new directions for research.

4 ELIGIBILITY CRITERIA AND EVALUATION OF THE LETTER OF INTENT

Eligibility criteria

- the letter of intent to participate in the proposed consortium must respond to the objectives of this call for projects and propose to **carry out at least 2 out the 3 tasks** described above;
- If the project emanates from a single team, the project coordinator must be a researcher or tenured teacher-researcher **working within an official Inserm team**
- If the project emanates from a consortium of teams:
    - the project coordinator must be a researcher or tenured teacher-researcher working within an official Inserm team, **and/or be employed by Inserm**
    - the other partner investigators of the consortium must hold a permanent research position in France
    - **if the coordinator him/herself does not belong to an Inserm team, then at least one partner investigator must be working within an Inserm unit**
- all leading partners must specify:
    - their time commitment to the project;
    - the resources, particularly in terms of staff or equipment that they intend to use as part of the cross-cutting program, in agreement with the responsible party from the partner institutions.
- a partner can be included into only one application (whatever the status: scientific coordinator of the project or partner within a consortium)

Evaluation criteria

After verifying eligibility, letters of intent will be submitted for evaluation by the scientific expert committee. Letters of intent not meeting the eligibility criteria will not be evaluated. The evaluation criteria are as follows.

Quality and originality of the proposed research

- Clarity of objectives and research hypotheses
- Innovative nature and advancement on the current state of the art

Skills/expertise

- Relevance of skills to the program objectives
- Possibility of combining skills within a broad network

Excellence of the research team(s)

- International recognition
- Skills of the team leaders within their discipline

Quality of the research environment

- Human resources to be used by the program
- Infrastructure available to carry out the program

Innovation/competition

- Innovative nature of the project in relation to international scientific issues or related to international competition

Expected added benefits

- Impact of added benefits in terms of knowledge and overcoming technological obstacles
- Role of the project in enabling the consortium to participate in international networks

5 ELIGIBILITY CRITERIA OF THE FINAL PROJECT

To be considered eligible, the final project must satisfy the following conditions:

- the project must respond to the objectives of the cross-cutting program;
- each working axis must include at least two teams with complementary skills, at least one of which must come from an Inserm research unit;
- the consortium coordinator must be significantly involved in the project.

6 PROGRAM CALENDAR

Date of publication of the call for projects		March 10, 2022
Opening of the project submission portal		March 10, 2022
Letter of Intent submission deadline	Electronic submission of the letter of intent	May 10, 2022
Meeting of the committee of scientific experts for the selection of the letters of intent		June 2022
Co-construction of work areas		July 2022
Seminar and presentation of work axes		September 2022
Deadline for submission of the finalized project	Submission of the project to Inserm	September 2022

7 OPERATING PROCEDURES OF THE CONSORTIUM

Coordination of the consortium

The coordinating institution of the consortium is Inserm. Inserm is responsible for implementing the chosen project within the cross-cutting program and, if necessary, formalizing collaboration between the partner institutions (public or private), by drawing up an agreement relating to the consortium, the production of project deliverables including the production of scientific reports, the organization of progress meetings, and the communication of results. The partner institutions designate the public or private entities in which the partner units are involved in the cross-cutting program. The partner units designate the research units, services, and teams involved in the fulfillment of the project and are placed under the responsibility of one or more partner institutions.
If non-Inserm partner units are involved in the consortium, they must have prior consent for their administrative supervision.

Duration of the project

Initial duration of the project is 3 years, with potential extension of 2 more years following evaluation.

Scientific reports

The scientific coordinator of the consortium will provide scientific reports to the coordinating institution according to the Charter of good practices and the procedures defined below.
They will be sent as per the following schedule:

- a progress report 6 months after the project has started;
- a report halfway through the project;
- a final report no later than 2 months after the end of the project.

The steering committee's scientific evaluation of intermediate and final scientific reports may lead Inserm to request additional information, suspend the project, or end financial support if the project is not being run properly or funding is being used for another project.

Responsibility of the scientific coordinator

The consortium scientific coordinator must inform Inserm and its partners, if necessary, via the steering committee, of any substantial modification of the research project or any difficulties hindering project completion.
The consortium scientific coordinator must also participate actively in the project monitoring procedures organized by Inserm (presentation seminars, colloquia, etc.).

Publications – communication

All publications resulting from the research project must include the following funding statement:

- **"Inserm cross-cutting program Mecacell3D 2022":** for English-language journals;

- **«Programme transversal Inserm Mecacell3D 2022»:** for French-language journals, press releases...

These publications are sent to Inserm for reference as soon as possible and at the latest five (5) days following publication.

Intellectual property

The results of the project belong to Inserm and the project partner institutions. The rules of ownership and the use of results from the project are defined as follows:

- between various partners in the context of a joint research facility: the applicable rules are those generally in force between the said various partners (in particular those of a joint research agreement);
- These rules will be defined in a separate consortium agreement between various partners associated with several research structures.

8 RULES FOR SUBMISSION

Submitting the letter of intent

The submission of your application involves a mandatory stage: registration on the Eva Inserm website and online submission of the letter of intent. This submission procedure, through the Eva Inserm website, includes:

- Providing candidate information (surname, first name, and email) enabling you to receive a user code and password giving access to a secure Eva personal space;
- uploading the letter of intent to the Eva website.
- **Submission deadline: May 10th, 2022**

You are strongly advised not to wait for the closing deadline before submitting your letter of intent.

Submission of the final project

This will be submitted to the coordinating institution, Inserm.

9 PUBLICATION OF RESULTS

The list of candidates selected from the letters of intent will be published on the Eva Inserm website.

10 CONTACTS

For any information, you can contact:

- For scientific and technical aspects: thematic institute BCDE, mecacell3d@inserm.fr
- For questions related to electronic submission: support.dsi@inserm.fr