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REPORT OF MEETING

3rd general meeting and working group meetings of the COST Action 16203: STEM CELLS OF MARINE/AQUATIC INVERTEBRATES: FROM BASIC RESEARCH TO INNOVATIVE APPLICATIONS (MARISTEM), December 3, 2019, METU-Culture and Convention Center (METU-CCC), METU-Ankara, Turkiye

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COST Action 16203 MARISTEM: state of the art and future perspectives

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In its first two years of life, the COST Action 16203 Maristem has organised two general meetings, two working group meetings, three workshops, two training courses, was present to one dissemination meeting and provided supports for 18 STSMs. Three additional workshops are forecasted within the 3rd GP.

Meantime, the number of full member countries increased from 20 (at the beginning) to 24, plus 1 COST cooperating member, 2 NNC and 1 IPC, with the involvement of 61 Institutions.

In the next two years, efforts should be directed towards the completion of the deliverables, the organization of new workshops and meetings aimed at deep discussions of themes of interest, and an increased exploitation of STSMs to support early career investigators.

A perspective of aging in botryllid ascidians

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Aging, the universal phenomenon ruled by the progressive degradation of organisms, has been the subject of high interest throughout the history of biological sciences. Almost all our knowledge on aging stems from studying solitary model organisms, while very little is known on aging of colonial organisms. It appears that studying colonial organisms may yield new understandings on aging that otherwise could not be studied in individual organisms. *Botryllus schlosseri*, a marine colonial invertebrate organism, is the subject of our study. In this colony three levels of structural organizations are assigned, each representing different forms of aging. The first level of organization are the zooids, the basic and the temporary units of the colony. The second level of organization is the whole colony, that is the sum of all zooids and the matrix they are placed in. The third level of organization is the entity that assembles several genetically different conspecifics (the chimera), due to morphological fusions between colonial vasculatures, a natural occurring phenomenon. On the zooid level, aging is developed synchronically once a week at all functional zooids, also characterized by an aging marker. At the second level of organization, the colony stage, 100 colonies were followed throughout their life span, revealing (still under analyses) that about one third of the colonies proceed through a natural fission process, and these genets were found to live significantly more than colonies that did not go through fission. This longer lifespan suggests an evolutionary advantage of the fission. At the level of the chimera, the study has been focused on chimeras between mothers and their offspring. Most of the offspring settled very close to their mothers (56 percent settle up to two centimetres from their mothers), leading to high prevalence of chimeras, resulting in fast degradation and death of the mothers compared to non-chimeric mothers. In contrast, chimerism between offspring resulted in a higher rate of growth and earlier onset of reproduction compared to the controls. These contrasting chimerism outcomes suggest for

divergent evolutionary trajectories. Thus, this study presents, for the first time, the uniqueness of aging in each level of biological organization in colonial organisms and opens a door for further studies in order to gain new understandings on aging.

New aspects in electron microscopy and *Hydra* stem cell lineages

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Hydra simple body is composed of three independent cell lineages, all of which contain large pools of either epithelial or small, set aside adult stem cells. They represent ancestral stem cell types present when animal multicellularity evolved. Epitheliomuscular stem cells of the ectoderm, which show features of undifferentiated and differentiated cell types, possess a vesicular trafficking system similar to bilaterians. In addition, the presence of large, in some cases huge, vacuoles implicate a function in transport of water and osmoregulation necessary to deal with a fresh water environment. Here, we have used scanning (SEM) and transmission (TEM) electron microscopy using high pressure-frozen tissue samples from the gastric region. After producing thick sections and SEM visualization, we found that the larger vacuoles connect via an elaborated canal system. This canal system could be connected to smaller vesicle types and larger macropinosomes dynamically forming at the apical epithelial membrane. Thereby, active intake and processing of particles of larger size is facilitated. Using ultra-thin section TEM, we are able to visualize the contractile actin structure, a dense network of actin monomeric filaments, building the macropinosome pores. Serial block-face scanning electron microscopy will be established in our lab, which will allow the detailed 3D reconstruction of this canal system and cellular ultrastructure in general.

Crosstalk between stem cells and differentiated tissues in planarians

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Stem cell fate depends on surrounding microenvironment, the so called niche. For this reason, understanding stem cell niche is one of the most challenging target in cell biology field and have to be unravelled by in vivo studies. Planarians offer this unique opportunity, as their stem cells, the neoblasts, are abundant, highly characterized and genetically modifiable by RNA interference (RNAi) in alive animals. However, despite impressive advances have been done in the understanding of planarian stem cells and regeneration, only a few information is available in defining signals from differentiated tissues, which affect neoblast stemness and fate. We took advantage of the stem cell repopulation process that follows low-dose X-ray treatment in planarians to identify genes, preferentially enriched in differentiated cells, whose expression is activated during the repopulation process. Silencing by RNAi of some of them impaired the stem cell repopulation, suggesting a tight extrinsic control of stem cell activity. Among these genes, we identified DjMAP that is expressed in the nervous system. DjMAP RNAi animals failed to regenerate thus indicating that it is involved in neoblast maintenance not only after low-dose X-ray treatment but also in untreated animals, thus paving the way for future studies in crosstalk mechanisms between DjMAP-positive neurones and neoblasts.

Stem cell molecular markers in the demosponge *Halisarca dujardini*

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Sponges are one of the most ancient multicellular animals that occupy key evolutionary position at the base of the tree of life. Many of metazoan-character signaling mechanisms and transcription factors appear in sponges. The appearance of stem cell systems should be studied from the simplest Metazoa. It was shown that genes participated in germ line program are expressed in different cell types of sponges like choanocytes and archaeocytes, but expression patterns differ from class to class of Porifera. We studied expression of myc (cell cycle regulator), pou (ortholog of oct4) and piwi (germ line associated RNase from Argonaute family) with whole mount in situ hybridization in demosponge *Halisarca dujardini*. All of these genes expresses in choanocytes. In addition transcripts are present in little patches of oocyte cytoplasm, by the structure corresponded to mesochyl cells ingested during oogenesis. These cells keep undigested in oocyte cytoplasm until cleavage begins. Perhaps presence of choanocyte-specific transcripts demonstrates that “nutrient” cells in oogenesis are dedifferentiated choanocytes.

Jellagen®: A next generation matrix derived from jellyfish for regenerative medicine and cell culture

A Mearns Spragg
Jellagen Limited

Jellagen® Limited is a marine biotechnologies company based in Cardiff, UK, whose strategic mission is to exploit sustainable marine species and natural resources, to develop technical and scientific high value research and medical device products, meeting state of the art specifications. Jellagen Ltd’s first range of products targeting cell culture include:

- Jellagen®: a next generation collagen biomaterial derived from jellyfish to support cell culture, tissue engineering and regenerative medicine applications.
- Jellagen®-2D: jellyfish collagen pre-coated plates – for improved Results in Promotion of Adhesion, Growth & Differentiation.
- Jellagen®-3D scaffolds: suitable for *in vitro* cell culture and tissue engineering

Jellagen Ltd’s research focus has led to the launch of a revolutionary new 3D cell culture

hydrogel technology; JellaGel™. Unlike mammalian collagen, JellaGel™ is free of disease vectors, non-specific miRNA and other contaminants typical of mammal-derived materials (*e.g.*, proteins, polysaccharides). Unlike synthetic materials, many of which are based on β-structured fibrous materials and produced chemically, jellyfish collagen is consistently bioresorbable and non-toxic to cells, from stem to lineage cells. JellaGel™ is able to provide and maintain a realistic, near-native microenvironments for cells. Encapsulation of cells has been shown to be synchronised with cell adhesion observed in all three dimensions, with cells showing no polarity as otherwise would be the case for 2D cultures. Biocompatibility has also been shown to be amply demonstrated by the appreciable expression of cellular filopodia within the hydrogel matrix.

Basic approach of inflammation, injury and regeneration in *Anemonia viridis*.

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The potential for tissue regeneration is a powerful adaptive strategy essential to the survival of individuals. It allows to face wounds or loss of body parts induced by predation, anthropic actions or environmental factors. In light of the high probability of increasing levels of disturbances caused by injuries and the increasing possibility of invasion of microbes and foreign agents in the tissues of anthozoans, it is crucial to determine how the species respond to wounds and physical damage and understand the capacity of recovering and tissues regeneration. From this point of view, the regeneration capacity of Anthozoa it could be considered an additional arm of innate immune defence and *viceversa*. Therefore, from our work team, the inflammatory response in Mediterranean anthozoan *Anemonia viridis* (Forsskal, 1775) has been studied following the injection of substances of various type and size. We observed strong and specific reaction, especially after the bacterial injection of *Escherichia coli* and *Vibrio alginolyticus.*

Then, we focused on the regenerative aspects of this species of anthozoan carrying out an experimental plan based on different numbers of tentacle cuts and the evaluation of the regenerative potential after 7, 14 and 21 days. Morphological observations and histological analysis on the tentacle regrowth, as well as measures of expression of proliferating cell nuclear antigen (PCNA) were carried out. Protease, phosphatase and esterase activities were measured as survival markers.

In perspective, we want to study at histological and molecular level how homeostatic tissues start the regeneration program while triggering immune response and their mediators of inflammation. In this context, we will focus on those bioactive molecules that, with their vast abundance, can be potentially used for biotechnological applications.

The enigmatic xenacoelomorphs: what they tell us about the evolution, development and regeneration of animals

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The emergence and diversification of bilateral animals are amongst the most important transitions in the history of life on our planet. However, although numerous labs are currently working on selected questions of genomic and morphological evolution, no interdisciplinary networks are currently in operation. As a result, many critically positioned animal groups have not been analysed and a comprehensive mapping of their genomes and tissue systems remains elusive. Our consortium brings together leading experts from different fields with the goal of elucidating the evolutionary and developmental origins of organismal and genomic complexity. Our aim is to provide answers to the questions: How did complex body plans arise in evolutionary time? How are complex body plans "encoded" in the genome? As the first step, we will focus on the earliest stages in bilaterian evolution, probing the most elusive organisation of the genomes and microscopic anatomy in basally branching taxa, which are currently assembled in a clade named Xenacoelomorpha. Our team's major long-term goal is to employ multidisciplinary approaches to decipher the genomic bases of the organisation and physiological roles of these organ systems. Moreover, we are now using these animal systems to study the regeneration of some key organs and tissues, a project facilitated by the enormous potential for regeneration of these organisms. I will describe in the meeting the progress we have made on the understanding of tissue architectures, the evolution of their genomes and the kinetics of regeneration in brain and gonadal tissues.

Shiok Meats - Cell-based clean shrimp meat

S Sriram, K Yi Ling
CEO and CTO, Shiok Meats Pte. Ltd., Singapore

Shiok Meats is a cell-based clean meat company, the first of its kind in Singapore and South-East Asia. Our mission is to bring delicious, clean and healthy seafood and meats by harvesting from cells instead of animals. Shiok Meats will bring cell-based crustacean meats (shrimp, crab, lobster) to your table. Our meats are animal-, health- and environment-friendly with the same taste, texture, more nutrients and no cruelty. "Shiok" in Singapore and Malay slang means fantastic and delicious. This presentation is our technology, mission, team and what we do at Shiok Meats.

Biosafety aspects for clinical applications of adult human mesenchymal stem cells

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Somatic cell therapy is a growing field of biotechnology and a promising alternative for the treatment of such conditions as graft versus host disease, digestive tract and inflammatory joint diseases, as well as cardiovascular and neurological diseases. Despite the potential, many problems are yet to be solved, including (1) the lack of sufficient body of clinical data and more thorough knowledge about in vivo post-transplantation processes; (2) donor-specific differences and heterogeneity of final cell product: (3) the variable functional activity of cells depending on post-transplantation microenvironment, (4) the inability of available animal models to accurately predict clinical outcomes in humans. Altogether, it implies many risks which result in inconsistent clinical outcomes. The main goal of biosafety is to mitigate risks and facilitate therapeutic efficiency which is achieved by manufacturing cell products in accordance with good manufacturing practice. One of the strategies for enhancement of therapeutic efficiency is cell product preparation according to individualized protocols, an approach chosen by Cilmes Sunu Tehnologjas, Ltd. to deliver patient-specific autologous therapeutics under the Hospital Exemption scheme (implementation of Art 28(2) of EU Regulation 1394/2007).