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Cell culture conditions: from outer space-like conditions to the mimicking of complex *in vivo* environments

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Dear Readers,

Imagine yourselves at the outer space, the void that lies beyond the uppermost reaches of the Earth's atmosphere, comprising all other objects in the universe[7]. Although it is a void, outer space may be thought of as an environment, where unprotected humans would perish. The principal environmental characteristic of outer space is the vacuum, where gas molecules are practically nonexistent and pressure is nearly zero, whose net effect on a human body would be unconsciousness and massive tissue damage in few seconds. The temperature range found in outer space also comprises a major obstacle, along microgravity and different types of radiation[7].

Getting trouble in wondering such completely different environment? The multiple Oscar laureate movie picture "Gravity"[4] can give us an astonishing picture of this scenario. Interestingly, with scientific accuracy the movie opening title card states: "At 600KM above planet Earth the temperature fluctuates between +258 and -148 degrees Fahrenheit. There is nothing to carry sound. No air pressure. No oxygen. Life in space is impossible". Obviously, the outer space exploration relies on scientific advances to mimic at outer space environment the minimal conditions that support human life, at least for a given time period.

Back from outer space reverie to 'regular' laboratory science, where usually we try to mimic at dishes, flasks and wells environments the minimal conditions that support cell life *in vitro*. As non-adherent cells, lymphocytes float in culture media similarly to humans at microgravity. Floating through a room/culture flask does not reproduce the standard routine of human and leukocytes, and consequently, impact the outcome of its biological process and responses. Indeed, it is more than clear that 'providing minimal conditions for maintaining cells alive' is not enough to fully mimick *in vivo* tissue environment[9].

Given the recent advances in the understanding of the mechanobiology of cellular functions[10], the necessity of reproducing *in vitro* the extracellular matrix derived mechanical support, and consequently resulting signals, became evident. Even in the case of adherent leukocytes, it has becoming clear that biological outcome of the adhesion in plastic surfaces differs from the adhesion in surfaces that resemble the extracellular matrix. Importantly, the raise and evolution of 3D culture systems seems to meet the expectation of researchers from different fields to improve *in vitro* systems. In a more ambitious endeavor, 3D printing of complex cellular scaffolds has experienced considerable improvement, not only in order to improve environmental cell experience *in vitro* but also in the translational transition to support tissue regeneration *in vivo*[2]. However, the classic to 3D culture system is still an evolving process, with numerous issues to be solved and questions to be answered.

Cell culture methods also have been improved in order to provide more realistic signals (in addition to the mechanical support derived signals) to mimic *in vitro* a series of different in vivo conditions. In a final parallel with outer space scenario, it is possible to compare the absolute absence of sound experienced by a human at outer space with the lack of soluble factors in culture media. In both situations, the absence of specific signals restrains the understanding of the surrounding environment and consequently influences a subsequent response. Indeed, cells are simultaneously exposed to a wide variety of environmental/exogenous (i.e. microbial products) and endogenous (i.e. growth factors, cytokines, hormones) signals *in vivo*, which collectively account for a final biological outcome. Note limited to physiological processes, the generation of additional signals due to an initial pathological process, can result in a differential outcome, such as a second pathological process, defined as a co-morbidity situation[3]. In oral science field, the development of diabetes and arthritis by previously healthy subjects (from the periodontal viewpoint), can trigger the development of periodontitis in response to the commensal oral flora[1,5,8,11]. However, the exact molecular mechanisms integrating different signaling pathways and their

impact to physiological and pathological processes remain to be fully elucidated.

In this context, in this issue of the Journal of Applied Oral Science, Medeiros, et al.[6] (2014) present an interesting study focused on the potential impact of different signaling pathways activation in the modulation of cell proliferation, survival and gene expression of T lymphocyte (JM) and monocyte (U937) cell lines. In the article, the authors investigated the effect of RAGE and TLR signaling, in order to mimic/reproduce *in vitro* the diabetes/periodontitis interaction scenario, were diabetes derived AGEs (which trigger RAGE pathway) and microbial derived LPS (which triggers TLR pathway) simultaneous signaling are supposed to account for the co-morbidity development. Interestingly, the authors found that there was no synergism between RAGE and TLR4 receptors on modulation of cell death and inflammatory gene expression in cell lines of innate and adaptive immune response, in contrast with previous observations.

The contrasting data presented by Medeiros, et al.[6] (2014) specifically reinforce the complexity involved in cellular response to multiple and simultaneous stimuli, but also demonstrate that relatively simple strategies for *in vitro* investigation (such as the combinatory analysis of multiple signals) can be useful in the study of complex *in vivo* scenarios, such as those underlying co-morbidities.

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