Final Report

CA 15214: An integrative action for multidisciplinary studies on cellular structural networks (EuroCellNet)

Inter-WG Meeting: Probing the effects of 3D Bioprinting Processes on Stem Cells' Functional Properties and Integrity

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Hosting Institution: LTFN-Nanotechnology Laboratory, Department of Physics, Aristotle University of Thessaloniki

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13 participants from 5 countries attended.

In his brief introduction, Prof. Yannis Missirlis from Mechanical Engineering & Aeronautics Dept. Laboratory of Biomechanics and Biomedical Engineering, University of Patras, Greece, gave an introduction to the meeting and a welcome address to the participants. In his talk he pointed out several important issues that the modern field of bioprinting is facing nowadays. He stressed on the fact that although current bioprinting techniques allow versatile, layer by layer production of complex tissue constructs composed of multiple cells and ECM components with high resolution, the effects of mechanical, thermal and other types of stresses the cells are exposed to during the process have not been thoroughly analyzed. Moreover, he called attention to the urgent need of discussing these issues together with detailed analyses on cellular fate during and after the process of bioprinting. He also highlighted the need of joint efforts of specialists in different fields of Biology, Chemistry, Physics and Engineering in order to successfully investigate and further be able to control the short and long term effects of the processing parameters on cell survival, DNA damage, as well as epigenetic and phenotypic characteristics in order to utilize this emerging tool affectively in clinical applications. He had mentioned some of the few examples addressing to these issues from the literature. With his talk Prof. Missirlis opened the floor for the talks of the other participants.

Prof. Michael Gelinsky shared their laboratory's experiences on 3D bioprinting in Centre for Translational Bone, Joint and Soft Tissue Research University Hospital

and Medical Faculty, Technische Universität Dresden, Germany, after giving a brief introduction to common additive manufacturing techniques. He had emphasized the problems of selecting the right material for 3D bioprinting, since the shape fidelity is highly lost as the cell viability is increased, and *vice versa*, using conventional techniques. Therefore, he emphasized on the need for development of novel strategies in order to preserve cell viability and functionality, while producing cell-material constructs with higher resolution and conformity. In this light, he presented the core/shell bioprinting technique, which according to their experience, allowed better shape stability of the material and at the same time better cell vitality. He introduced a novel synthetic nanoclay (Laponite), and showed their successful applications with alginate/methylcellulose/laponite composite gels that are stabilized by Ca^{2+} for adipogenic, chondrogenic and osteoblastic differentiation of human mesenchymal stem cells (MSCs). Furthermore, he presented some of his recent results with 3D bioprinting of microalgae, which they call “green bioprinting”. In his last minutes of talk Prof. Gelinky drew attention to several issues and yet unanswered questions in the field of bioprinting, which were later on during the Discussion panel conversed and debated among participants.

Prof. Aylin Sendemir-Urkmez from Biomaterials and 3D Biointerphases Group of Bioengineering Department, Ege University, Izmir, Turkey, pointed out the advantages and disadvantages of different 3D bioprinting techniques including extrusion, inkjet and laser assisted bioprinters; and gave examples from their 3D bioprinting experiences on skin, vascular and osteochondral tissue engineering. She discussed on the requirements for successful "bioink" properties and "printability" for each printing technology, and use of "support matrix" during bioprinting. She presented the ongoing projects in her lab with bioprinting and ended her talk with critical points that should be analyzed and discussed by all scientists in the field of bioprinting.

Prof. George Miloshev from Laboratory of Molecular Genetics, Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria, emphasized the probable effects of 3D bioprinting process on gene expression and spatial organization of the genome, and possible genotoxicity caused by the process. He informed on novel techniques for determination of such effects, as well as shared his lab expertise in the development and application of novel techniques for single cell analysis of cellular viability, genome stability and epigenetic make-up, which could be used in the studies of cellular fate before, during and after the process of bioprinting

Prof. Mara Grube from Institute of Microbiology and Biotechnology, University of Latvia, Riga, Latvia, suggested FT-IR spectroscopy as a powerful quantitative technique to access stem cell differentiation and other possible distinct effects of processing parameters during 3D bioprinting, giving examples from their own experience.

After lunch and introduction of each participant present in the meeting, a three-hour long discussion and brainstorming session took place. The main challenges and questions raised on success of the 3D bioprinting were:

1. What types of cells should be used in 3D bioprinting? Is the process applicable for all types of cells?
2. Shall bioinks encapsulate cells or allow their migration?
3. How should cell/MSC differentiation be evaluated after bioprinting?
4. How does shear and heat created during the process affect cell viability and differentiation? And moreover, how could such stress be evaluated?
5. What are the applicable methods for quantification of cell numbers, matrix components and differentiation markers when cells are embedded in hydrogel matrices?
6. How could the lack of proper microscopical characterization due to thickness of bioprinted multilayer constructs be overcome?
7. How could we solve the yet unsolved problem of bioink design and subsequent gel rheology that facilitates bioprinting of macroscopic & mechanically stable constructs?
8. Is it possible to overcome the low cell proliferation observed in 3D bioprinted constructs - the difficulty to verify material aspects versus stress effects?
9. And as Ca^{+2} concentration (used for stabilization of alginate bioinks) can be an important concern for cellular behavior, how could we possibly solve this problem?

The suggestions, recommendations and future aims in the field of successful 3D bioprinting that were reached collectively during the meeting in order for novel approaches and collaborative proposals to be developed were as follows:

1. Design and development of standardized protocols for mixing cells with bioinks is an urgent task. There is need for standardized protocols for initial mixing of cells in bioink, and the time in the nozzle, since O_2 concentration might also be a critical factor as well as shear stress;
2. Standardized protocols for evaluation of cellular viability in the 3D printed structures are major prerequisites for the future success of the technique;
3. Epigenetic profiling of cells before and after mixing in bioink, as well as out of the printer and after a relevant culture time is essential for conclusive results;
4. Epigenetic markers of stress must be identified in order to evaluate processing effects;
5. Core/shell bioprinting and combinations of different biomaterials within the same bioprinted construct can be a possible approach to combine cellular viability and construct stability;
6. FT-IR can be used to detect specific protein modifications during and after bioprinting applications, and could be standardized as a technique in 3D bioprinting evaluation of cellular fate;

7. For relevant clinical applications, stem cells are more likely to be the starting cells; and their stemness must be qualified before and after the bioprinting process;
8. "Green bioprinting" - biopriting of algea with mammalian cells - was suggested as a new concept that can provide O_2, and elongate the critical time required for *in vivo* neovascularisation of tissue engineered constructs.
9. Incorporation of online monitoring of local O_2 concentration and other relevant physical and chemical parameters into the culture and/or bioink systems would be extremely beneficial to control cellular behavior throughout the process and to determine important drawbacks.