Available online at www.sciencedirect.com

ScienceDirect

Procedia CIRP 49 (2016) 174 – 177

www.elsevier.com/locate/procedia

The Second CIRP Conference on Biomanufacturing

TISSUE SPHEROIDS ENCAGED INTO MICROSCAFFOLDS WITH INTERNAL STRUCTURE TO INCREASE CELL VIABILITY

J.A. Dernowsek [a,*], R.A. Rezende [a,*], V.E. Passamai [a], P.Y. Noritomi [a], D.T. Kemmoku [a], J.A. Nogueira [a], V.F. Lara [a]
V. Mironov [a,*], J.V.L. da Silva [a]

[a]*Center for Information Technology Renato Archer (CTI), Division of 3D Technologies (DT3D), Rodovia D. Pedro I (SP - 65) Km 143,6, Amarais,* Campinas, SP, 13.069-901, Brazil

* Corresponding author. Tel.: +55-19-3746-6203; fax: +55-19-3746-6204. *E-mail address:* janaina.dernowsek@cti.gov.br

Abstract

Aggregates of pre-sorted cells form structures called tissue spheroids that have been widely used in the field of tissue engineering. The greater contact of the cells with the culture medium is directly related to cell viability and to the increase of proliferation rate. Due to the characteristics of a 3D environment, at some zones within the tissue spheroids some cells are not equally exposed to the environment, impacting in the formation of microenvironments with decreased oxygen, nutrients and soluble factors produced by cellular metabolism leading to the formation of low proliferation areas and consequently necrosis (cell death). The fusion of cells also changes the catabolites flow, generating a very heterogeneous diffusion. The idea of this project is to develop and improve a microscaffold based on the concept of lockyballs that have cell support function for tissue engineering. Lockyballs have hooks and loops which attach each other assuring a 3D cellularized mesh. The model has a spherical outer structure and inner hollow microsphere constituted by pores with diameters smaller than the cell ones, whose function would be to prevent cell entry and provide an environment suitable to diffusion gradient necessary for the cell viability of the spheroid avoiding necrosis. The first stage of the work consisted in the generation of different three dimensional models by Computer Aided Design (CAD) software Rhinoceros 5.0. At the second stage, the CAD model was imported into finite element method (FEM) software (ANSYS R16 Static) to perform computational simulation, which consisted of structural analyses. Computational simulations were essential to predict the diffusion phenomenon inside the whole 3D structure. The development of new microscaffolds models can enhance the regenerative capacity and 3D tissues construction.

Peer-review under responsibility of the scientific committee of The Second CIRP Conference on Biomanufacturing

Keywords: Tissue Spheroids; Viability Cell; Computational Simulation; Microscaffold; Tissue Engineering; Lockyballs

1. Introduction

The cell culture is characterized by allowing maintenance interaction of living cells in the laboratory, independently of the body from which it was originated, and its use allows a better understanding of the molecular mechanisms of the cell, thereby enabling scientific breakthroughs. The cell culture on three-dimensional (3D) initially derived from commonly used culture cells (monolayer) whose differential 3D culture is to allow cells exploiting the 3D space, increasing interaction with the environment, between cells and the extracellular matrix [1]. The greater contact of the cells with the culture medium is directly related to cell viability and increased proliferation rate and that, due to the characteristics of a 3D environment, cells are not equally exposed to the environment. This fact means that there is the formation of microenvironments with regionalized decrease of oxygen, nutrients and soluble factors produced by cellular metabolism leading to the formation of low proliferation areas and consequently necrosis (cell death), so the cluster of cells also changes the catabolites release flow, making your very heterogeneous diffusion and these changes directly affect the cellular physiology of the spheroid [2, 3].

According to Montel *et al.*, 2011 [4], the effect of a mechanical stress on the long-term growth of a spheroid cell aggregate affects the growth by inhibition of cell proliferation mainly in the core and their results demonstrated that a stress

Peer-review under responsibility of the scientific committee of The Second CIRP Conference on Biomanufacturing
doi:10.1016/j.procir.2015.11.010

of 10 kPa is sufficient to drastically intervene in the cell viability. However the results showed that the mechanical stress transmitted to the spheroid surface is reversible, meaning that once the pressure is released, the spheroid can grow again.

Its also knows that the osmotic stress has direct effects in cell proliferation, cell migration and apoptosis, through the mitogen activated protein kinase (MAPKs) pathway [5].

Helmlinger *et al.*[6], in 1997 observed that the basis of general mechanism for cell mechanosensitivity, the influence of mechanical stress exerted by environment was demonstrated in a pioneer *in vitro* work on spheroids embedded in agarose and the results indicated that a range of 45 – 120 mmHg (5 – 15 kPa) modulate the cell growth. In a more recent work, Loessner et al.[7], in 2013 compared a mathematical model with the experimental output about the influence of mechanical and biochemical stimuli in the growth of multicellular spheroids and their results demonstrated that it is possible to simulate solid tumor growth on the basis of data on spheroid size, cell proliferation and cell death within these spheroids.

Due to the increasing number of works involving tissue spheroids, the search for more knowledge is steadily increasing and understanding the three-dimensional biomechanical properties is crucial. There are several suggestions already validated and some still not validated assumptions, devices or arguments that taken together constitute a conceptual basis for issue spheroids approaches in tissue engineering [8]. Rezende *et al.* [9], 2012 approached the design, physical prototyping and the use of an interlockable solid microscaffold termed "lockyball" for tissue self-assembly for biofabrication uniting the concept and use of microscaffolds and tissue spheroids.

Despite intensive publications on this subject, still remains one of the least understood topics in developmental biology and the ultimate applied goal of regeneration biology is to develop methods to improve regenerative capacity and 3D tissue construct.

To bridge the gap, several methods, devices and techniques have been developed to improve the O_2 and CO_2 diffusions, the transport of nutrients and cellular waste, to reduce the mechanical stress and increase the number of cells in the spheroid. The idea of this project is develop and improve models of support, similar to those already developed in DT3D - Three-Dimensional Technologies Division of CTI, such as "Lockyballs", which have the cell support function for tissue engineering. As an innovation, the model has beyond the outer spherical structure, inside a hollow microsphere constituted of pores with diameters smaller than cell, whose function would be to prevent cell entry and mimic an environment suitable to significant diffusion gradient necessary and important for the inner cell viability of the tissue spheroid. Below this propose, our work aim to create a new concept of microscaffolds to spheroids in order to prevent the mechanical stress induced by the microenvironment. Thus, we hypothesized that tissue spheroids with solid microscaffolds in certain case non-biodegradable biomaterials could enable the rapid *in vivo* biofabrication of 3D tissue constructs at desirable material properties and high initial cell density, and improve diffusion of nutrients (figure 1). Recently, biocompatible photo-sensitive biomaterials could be fabricated at nanoscale resolution using two-photon polymerization (2PP).

Hence, our strategy will help to study which mechanisms may affect the properties of the spheroids during 3D tissue construct and the 3D cell culture.

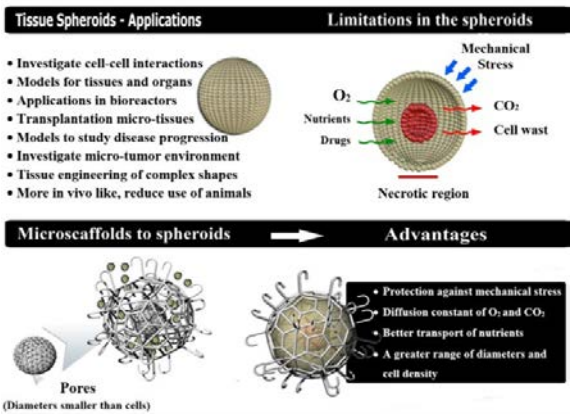

Fig. 1. Limitations and applications of the tissue spheroids and advantages of new microscaffolds with internal structure.

Methods and Materials

Solid microscaffolds with internal structures have been designed and modeled using preliminary design studies finite element analysis software and in the future they could be fabricated using two-photon polymerization of organically modified photo-sensitive biomaterials according to the original lockyballs [9].

The complete microscaffold structure was modeled in the Rhinoceros® 5.0 (McNeel North America, Seattle, WA, USA) software, and the '.iges' file was imported into Ansys 16.0 (ANSYS Inc, Houston, TX, USA) for the finite element analysis (FEA). All materials were considered isotropic, linear, homogeneous and their moduli are presented in the Table 1. No consensus exists in the literature regarding the mechanical properties of the materials. Thus, the most frequently reported values were used. The contact regions between the structures were considered perfectly bonded, and the mesh, with a total of 6025 nodes and 3249 elements. The base of the microscaffold was considered fixed in six nodes, and the base of the sphere (cell aggregate) was considered fixed in one node.

Table 1. Mechanical properties of materials used on Finite Element Analysis.

Material	Young 'modules	Reference
Ormocer®	678 MPa	-
Alginate	0.18 – 20 kPa	[10]
Cell aggregate	~ 50 Pa	[11]
Single cell	~ 40 - 50 Pa	[11]

Most of the models developed up to now studing tissue spheroids (cell aggregates) behavior are concerned with aspects fluid-dynamic considerations using several software such as ANSYS® CompuCell3D®, MatLab®, SimLab, Surface Evolver, among others and in the present work a structural approach was considered (figure 2). The CompuCell3D is a software for simulating environment for multi-cell, single-cell-