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Human bioidentical ECM expression for a new era of bioinputs

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Introduction

Review and Revolution

Quantis' vision is to develop a sustainable and totally animal free human extracellular matrix biofabrication process through fibroblasts and chondroblasts cell cultures, 3D bioprinting a construct (our newly developed Quantum Tissue platform) and extracting inputs such as collagen types I and II, elastin, and fibronectin. Since there are no other extracellular matrices (ECM) free of animal sources and human bioidentical available in the global market, these and other green inputs we produce are capable of revolutionizing the R&D market. There are some human-derived collagen today, but they are much more expensive than the animal-source alternatives and don't have other matrix components, besides having low concentrations of collagen. Our main idea is to provide a revolutionary extracellular matrix at the same time as we break up with the animal input use pattern, developing a brand new method of production, unique in the world.

The first of our inputs is collagen with high purity and bioidentical to humans. This choice was driven both by the different properties of the input obtained by our process and by a production efficiency superior to other methods of obtaining this type of input. Besides the ECM extracted by human fibroblast monolayers, there are synthetic matrices in the market willing to compete with other complex ECMs, offering similar results at a more affordable price point, compared to the monolayer matrix. Today, the most commonly used collagen source is the rat tail, since it is the cheapest alternative and has lots of researches and methodologies developed in the last decades. Quantis's primary objective is to be the first

choice at the ECM market, bringing a range of proteins within an attractive price range, aiming to replace animal collagen in the next few years.

Quantis ECM's superior efficiency is the result of our production process, using frontier technologies such as bioprinting and genetic engineering. In our 3D production system,cells are attached to a matrix, which guarantees a higher yield of ECM production than if it was not in this condition. The 3D matrix (i.e., 2D versus 3D) also plays a key role in determining how a cell will respond to biochemical and mechanical cues, since in many native tissues cells are completely surrounded by ECM. Conventional 2D cell culture has provided important insight into how cells interact with their environment, but there is increasing interest in creating more physiologically relevant 3D cell culture models in order to study cell-matrix interactions and their effects on cell function (HONG H, 2008)[1]. Thus, we estimate that an innovative 3D system will be capable of producing matrix proteins up to 20 times greater than current methods.

Speaking of collagen sources, the most common, reviewed by JM Lee et al. (2021)[2] are:

> **Salt-soluble collagen:** extracted using a cold neutral salt bath, which imposes a limit in the yield and purity of the final solution.
>
> **Acid/ enzyme soluble collagen:** different types of collagen-rich tissues are immersed in a solution (acid or enzymatic) to degrade the initial tissue and release the collagen proteins. Currently, researchers are investing in collagen derived from marine sources due to some advantages over mammal-derived collagen, such as ease of extraction, better durability (chemical and physical) and abundant availability. However, the final result is a collagenic solution with fragmented or peptides of collagen, without any other matrix components.

Recombinant collagen: proteins expressed by prokaryotic and eukaryotic host systems, most commonly fibroblasts and chondrocytes (expressing type 1 and 3 collagen, respectively). This type of collagen source reduces batch-to-batch variation, interspecies disease transmission and xenogeneic immune responses. In bacteria, the absence of hydroxyproline lowers the stability of collagen triple helix, affecting fibrillogenesis. Plants are capable of synthesizing hydroxyproline, resulting in a protocollagen. Unfortunately, the yield of production is generally low, limiting a potential commercialization.

Synthetic collagen: collagen-mimicking peptides that bear the trimeric Gly-X-Y sequence, allowing the control of physicochemical properties to suit intended applications. However, these peptides contain only 15 to 40 peptides, making the achievement of gelation and self-assembly harder than with other sources.

Quantis was born to create a new kind of collagen source, unifying concepts of correlated areas such as biotechnology, genetic engineering, bioprinting and molecular biology. Our main goal is to establish a unique production method, providing human extracellular matrix components to the world with competitive prices, helping in the development of aesthetics and in vitro tissue engineering.

The purpose of this paper is to compare the current production methods of collagen and extracellular matrix from human sources, mainly by fibrosarcoma/ kidney epithelial cells in monolayers and/ or in spheroids.

The various methods of collagen expression

Quantis predecessors in biofabrication

Expression of Procollagen by Fibrosarcoma (Geddis, Prockop. 1993)[3]

Back in 1993, in Philadelphia, Amy Geddis and Darwin Prockop developed a recombinant system of collagen expression using HT1080 cells transfected with a construct containing the cDNA for the human COL1A1 gene under the transcriptional control of the promoter and enhancer of the immediate early gene of CMV and a neomycin-resistance gene.

According to the results related, about 2% of the original 600 G418 resistant clones secreted relatively large amounts of homotrimeric type I procollagen, and the authors hypothesized that the site of transgene integration into the host cell genomic DNA may have served to silence the transgene expression. Also, concatemerization, rearrangement and point mutation of the transgene during the process of transfection and integration seem to explain the small number of synthesis of collagen by a small number of transfected cells.

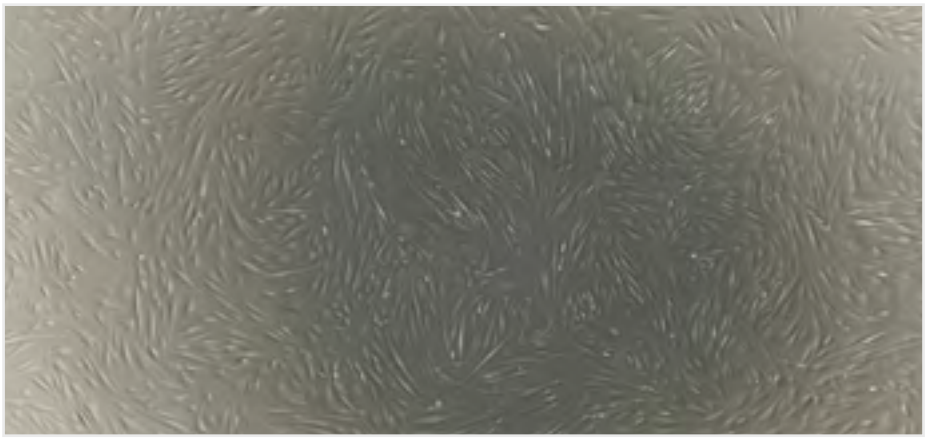
This study has shown the world that it was possible to express collagen using fibrosarcoma transfected cells, even with low yield of expression and production. However, after almost 3 decades, researchers and companies have not optimized this method of collagen production, implying that it isn't commercially viable even with modern techniques.

Expression of collagen by human hepatic stellate cells (HSCs) (Smith-Cortinez et al. 2020)[4]

A recent study conducted in the Netherlands around liver fibrosis showed that it is possible to express type I collagen by HSCs treated with TGFβ, having the ascorbic acid required for a more effective release system.

Without the action of the ascorbic acid, most of type I collagen produced stay in the intracellular dependences, requiring a further extraction method. Human primary HSCs did not increase αSMA protein or mRNA expression after TGFβ treatment, suggesting that p-hHSCs were already activated after in vitro culturing.

Even with this non-orthodoxal example of expression, it's highly unlikely that we will see a commercial application of this type of collagen production in the near future, since currently the reagents and culture medium to maintain this kind of cells are very expensive and not scalable.



Typical cellular culture proliferation.

Source: Quantis Research & Developement

Expression of collagen by silicotic animals fibroblasts in 3D cell culture (Silva et al. 2012)[5]

In this doctoral thesis, fibroblasts from animals with silicosis (a restrictive pulmonary disease caused by the inhalation of crystalline silica particles, which is characterized by chronic inflammation associated with intense fibroblast proliferation and exacerbated accumulation of extracellular matrix components) were used to establish a system of primary cell culture in 3D, spheroids using lung fibroblasts from normal and silicosis mice.

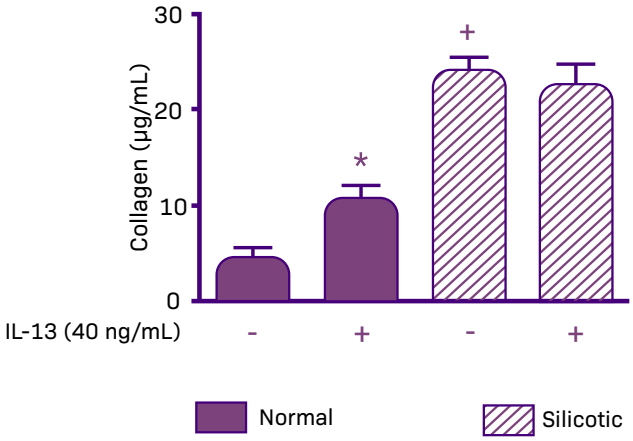


Figure 1: Quantification of collagen in the supernatant of spheroids containing pulmonary fibroblasts obtained from normal and silicotic mice. Analyses were performed 3 days after stimulation with IL-13 (40 ng/mL) in vitro, through inverted light microscope (4x magnification). Source: Silva (2012)[5]

Due to the team's work, it was possible to prove that fibroblasts from silicotic animals would be bigger and denser if compared to the normal animals. This reinforces the idea of activation conditions of these cells represented by the increase in cell proliferation and the production of extracellular matrix elements, as shown in the figure 1.

This thesis shows that it is possible to create a high-yield mechanism of extracellular components expression. However, it would use mice fibroblasts, making it impossible to use in the field of aesthetics. Thus, the extraction and purification of this extracellular matrix could potentially damage collagen protein structures, resulting in fragments and peptides.

Expression of ECM by Quantis

The beggining of the biofabrication new era

In the introduction of this Whitepaper, some types of collagen expression were addressed, such as salt-soluble, acid/enzyme soluble, etc. Understanding the current methodologies and wanting to improve the world's in vitro development, Quantis has created a fully-new pattern of collagen and other ECM proteins expression using an innovative work flow.

In an abstract, Quantis methodology is described in Figure 2, with the 4th step being the revolutionary expression method patented by the company. Currently, many companies and researchers are used to work with 3D constructed tissues for further development and analysis. However, only Quantis has a procedure that uses this 3D bioprinted tissue as a real biorreactor (Figure 3). **With this, human fibroblasts are capable of reaching its maximum in vitro yield of production and expression of extracellular matrix, since it would have a conformation such as a protoskin.**

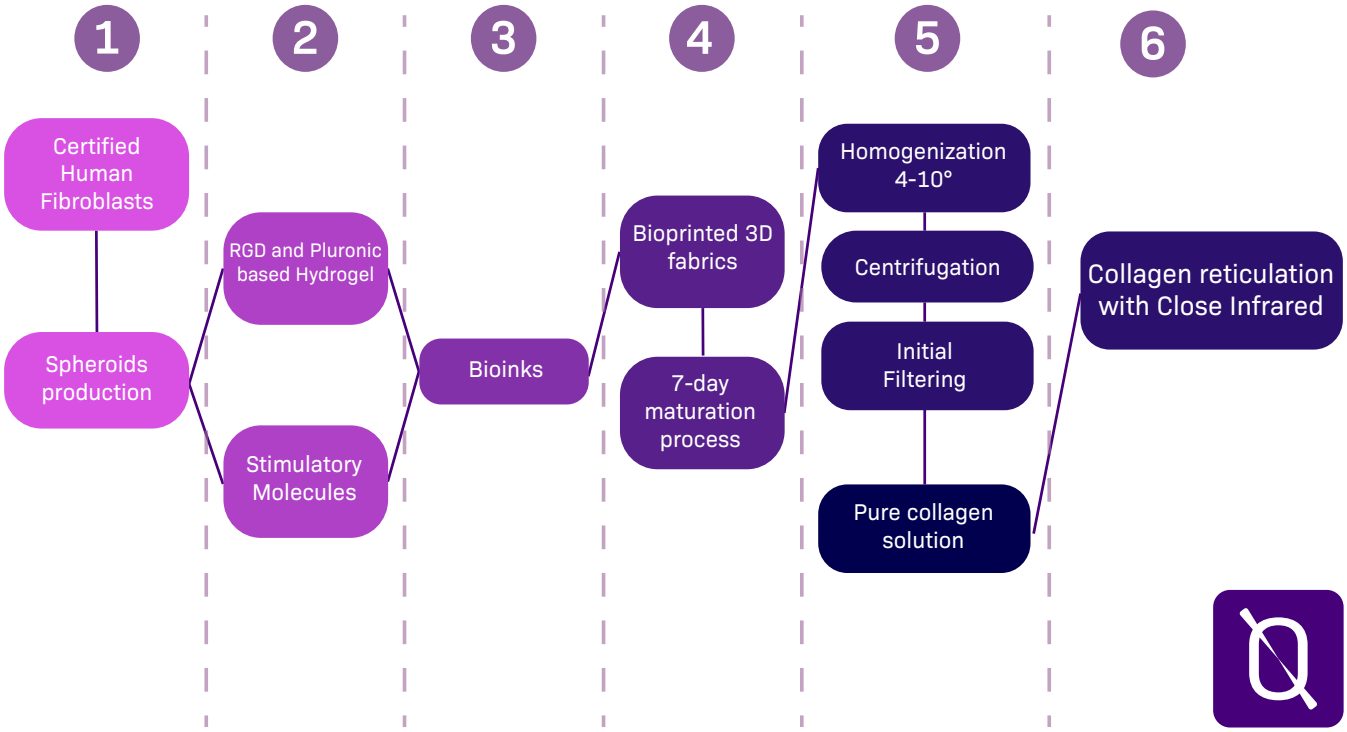
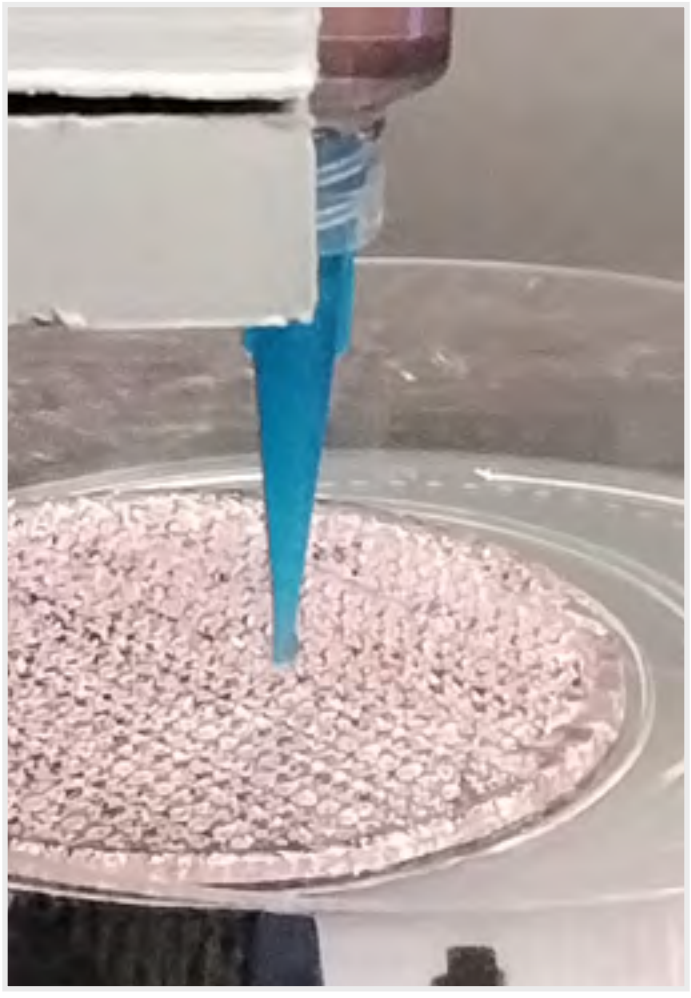


Figure 2. Quantis production process flow divided into 6 main steps: (1) 2D cell culture and spheroids production, (2) hydrogels and stimuli, (3) bioink, (4) bioprinting, (5) extraction of collagen and analysis, e (6) collagen photoreticulation strategy. Source: own image.

Figure 3: 3D bioprinted tissue in the Quantum Tissue bioprocess (Quantis ™).



To reach the 3D construct that we call Quantum Tissue there must be a pattern in the spheroids number in each microwell in order to have a better viability in the bioink formulation. The advantage of working with spheroids (3D structures) is that the cells are already programmed to organize themselves in three dimensions and, consequently, to increase the production of extracellular matrix (Duval et al., 2017)[6].

Figure 4 shows the relation between the diameter of the spheroids and the number of present cells. From these results, 500 cells will be continuously used per well to obtain spheroids with approximately 250 µm in diameter and with more than 80% viability.

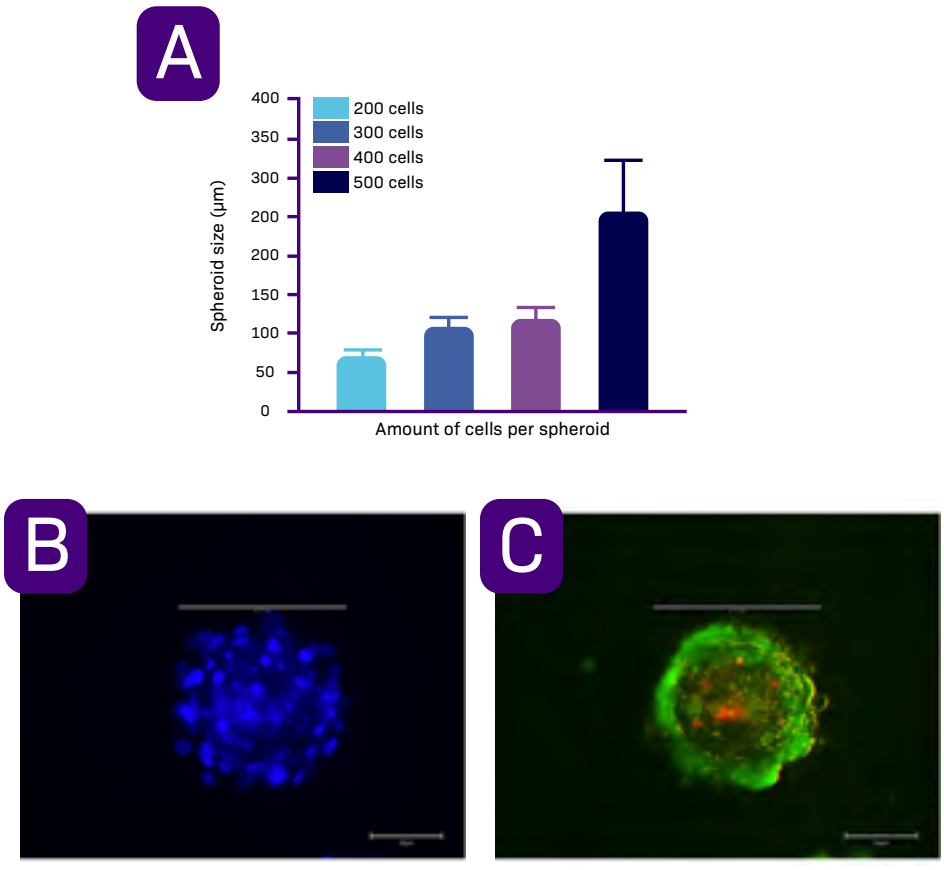


Figure 4: A) Graph showing the relationship between the number of cells per spheroids and the size of the spheroids. B) Spheroid nuclei marked blue by Hoescht dye. C) Live.Dead assay containing spheroid with viable cells marked in green staining and non-viable in red staining. Scale: 50 um. Source: Own image.

Quantis' Minimum Viable Product (MVP)

Safe, scalable and human

After almost 2 years of development, with setbacks due to the global pandemics, the first batch produced was bottled and subjected to biochemical and biological analysis, being sent to the first companies to close partnerships with Quantis. As preliminary results, it was observed that our product QMatrix was not toxic to cell culture, free of bacterial and fungal contaminants, and was able to stimulate fibroblast increase and proliferation at a concentration of 80 ug/mL after 48 hours of treatment, as shown in Figure 5.

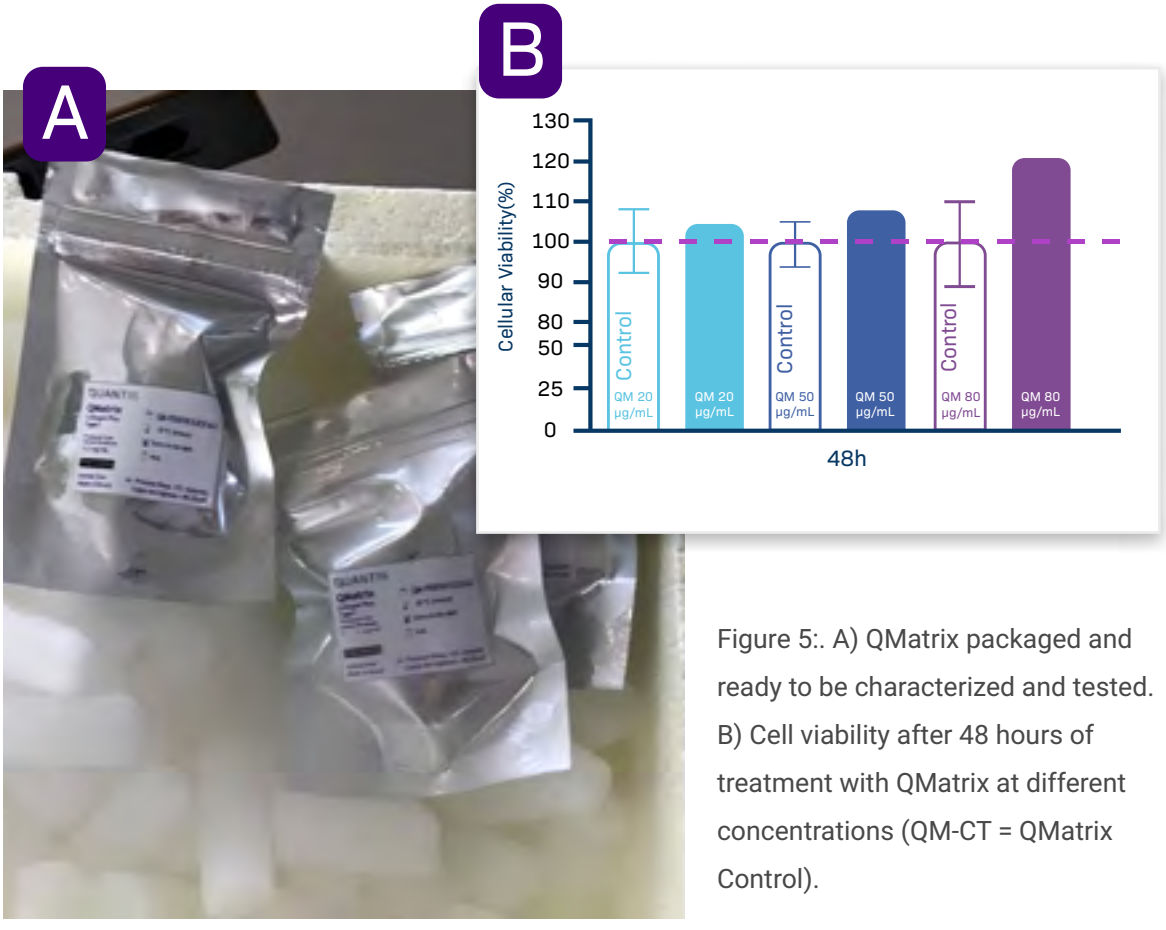


Figure 5:. A) QMatrix packaged and ready to be characterized and tested. B) Cell viability after 48 hours of treatment with QMatrix at different concentrations (QM-CT = QMatrix Control).

This initial result shows the potential impact QMatrix would have on the in vitro development. A 2008 study conducted by SERBAN M, LIU Y and PRESTWICH G in the University of Utah7 did a comparison study with fibroblast, using 3 different complex extracellular matrices. The results are shown in Figure 6.

Using a double straining microscopic evaluation, the cell viability of the fibroblasts in all hydrogels was estimated to be 85-90%. The main goal of this study was to establish data to offer a rational basis for selecting and optimizing an ECM equivalent for specific 3D culturing experiments.

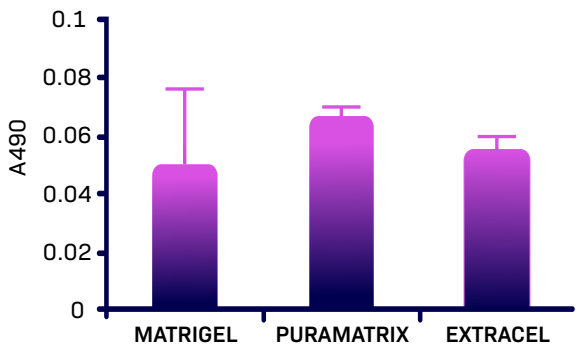


Figure 6: Proliferation rates of fibroblasts encapsulated within various ECM analogues. Columns represent mean ± SD, n=4, p>0,05. Source: https://doi.org/10.1016/j.actbio.2007.09.006.

Conclusions

With a high cell-viability even in low concentrations, QMatrix has proven to be an optimal competitor for 3D cell culture and in vitro replications. Additionally, due to the presence of physiologically human protein-structures in the solution, Quantis' product has lots of potential in the regenerative medicine market. The company's next steps and focus are:

Reduce batch-to-batch variations;

Further quantifications of the elements present in the solution;

Create a portfolio of usage with national and international partners;

Establish a growing commercial tie with many companies.

References

1: HONG, Helen; STEGEMANN, Jan P. 2D and 3D collagen and fibrin biopolymers promote specific ECM and integrin gene expression by vascular smooth muscle cells. Journal of Biomaterials Science, Polymer Edition, v. 19, n. 10, p. 1279-1293, 2008.

2: LEE, Jia Min et al. Bioprinting of Collagen: Considerations, Potentials, and Applications. Macromolecular Bioscience, v. 21, n. 1, p. 2000280, 2021.

3: GEDDIS, Amy E.; PROCKOP, Darwin J. Expression of human COL1A1 gene in stably transfected HT1080 cells: the production of a thermostable homotrimer of type I collagen in a recombinant system. Matrix, v. 13, n. 5, p. 399-405, 1993.

4: SMITH CORTINEZ, Natalia et al. Collagen release by human hepatic stellate cells requires vitamin C and is efficiently blocked by hydroxylase inhibition. The FASEB Journal, v. 35, n. 2, p. e21219, 2021.

5: SILVA, Andressa Moraes Guimarães da et al. Avaliação da reatividade de fibroblastos provenientes de animais silicóticos: estabelecimento de sistema de cultura em 3D. 2012. Tese de Doutorado. Instituto Oswaldo Cruz.

6: DUVAL, Kayla et al. Modeling physiological events in 2D vs. 3D cell culture. Physiology, v. 32, n. 4, p. 266-277, 2017.

7: SERBAN, Monica A.; LIU, Yanchun; PRESTWICH, Glenn D. Effects of extracellular matrix analogues on primary human fibroblast behavior. Acta biomaterialia, v. 4, n. 1, p. 67-75, 2008.