Research Dynamics of Tissue Spheroids as Building Blocks: A Scientometric Analysis

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Abstract: Tissue spheroids represent an innovative solution for tissue engineering and regenerative medicine. They constitute an in vitro three-dimensional cell culture model capable of mimicking the complex composition of a native tissue on a micro-scale; this model can function as a building block and be assembled into larger tissue constructs. Due to the potential tissue spheroids have for the evolution of the health industry, there is a need to assess the research dynamics of this field. Thus far, there have been no studies on their use as building blocks. To fill this gap, a study was performed to characterize the evolution of research where tissue spheroids were used as building blocks to generate tissue constructs. A scientometric analysis of the literature regarding tissue spheroid technologies was developed by quantification of bibliometric performance indicators. For this purpose, articles published during the period January 1, 2015 – December 31, 2021, from the Scopus database were organized and analyzed. The main subject areas, countries, cities, journals, institutions, and top-cited articles as well as the types of techniques, cells, culture time, and principal applications were identified. This research supports the definition and growth of research and development strategies for new technologies such as tissue spheroids.

Keywords: Tissue spheroids; Biofabrication; Bioassembly; Bioprinting; Scientometrics; Technology intelligence

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1. Introduction

Tissue spheroids are three-dimensional (3D) organized clusters of cells that mimic the cellular compositions of tissues[1]. They provide intensive cell-cell contacts, outstanding regenerative properties, and rapid growth useful for high-throughput production. Tissue spheroid technologies can be structured at a cellular level in native tissue and can be assembled into larger structures, preserving the characteristics of native tissue without the constraints of scaffolds[2-4].

Tissue spheroids can represent the nature of tissue on a micro-scale, serving as a model for drug testing research or functioning as building blocks[1,5]. Tissue spheroids as building blocks have gained the attention of academia and industry, as they can generate customized tissues without the use of scaffolds. Thus, tissue spheroids could mitigate the lack of donors for tissue transplantation by generating tissue constructs that can function properly in terms of cell differentiation and proliferation[6].

Within this context, there are two methods for generating tissue spheroids. In the seeding method, tissue spheroids can be injected or seeded directly. Regardless of the structural limitations of the final tissue construct, seeding method is the most widely used approach because it does not require sophisticated tools for the placement of tissue spheroids. The alternative method is biofabrication, which is defined as the automated production of a biologically functional construct that incorporates living cells, bioactive molecules, and biomaterials[7]. Biofabrication encompasses bioassembly and bioprinting techniques, which are automated processes that enable the generation of two-dimensional or 3D structures, based on a pre-defined pattern or structure[7,8]. The techniques
differ in terms of the minimum required fabrication unit size: Bioprinting is performed at the molecular level, while bioassembly is performed at the cell cluster level[9]. Compared with the seeding method, the biofabrication method enables greater control over the final design of the tissue construct, allowing the production of complex geometries and precise distribution of cell clusters.

Bioassembly utilizes different techniques to develop tissue spheroids as building blocks, including Kenzan[9], Bio-Pick, Place, Perfuse[10,11], and magnetic-directed patterning[12,13]. Within the bioprinting framework, there are two techniques for developing tissue spheroids: inkjet bioprinting[14] and extrusion-based bioprinting[13].

The aim of the current study is to evaluate and define the current status of tissue spheroids as building blocks. For this purpose, we applied scientometric tools[16,17]. According to Hess[18] scientometrics involves the “quantitative study of science, communication in science, and science policy.” While scientometrics at first was predominantly manual, now this field has evolved to a level where text mining techniques are used to analyze different information sources, which include peer-reviewed scientific publications, web sites, and social media (the latter being the newest). Scientometrics adds value to decisions related to research and development (R&D), innovation, and in general technology management serving academia, industry, and government. The research dynamics of a field can be determined through the analysis of bibliometric indicators where the growth of fields, technology trends (leading countries, cities, authors, institutions, and journals…) as well as the focus of research and the newest advancements can be determined.

To identify the research dynamics of tissue spheroids as building blocks, this study quantified bibliometric indicators related to publishing performance: Number and growth of publications; predominant subject areas; kinetics of growth from 2015 to 2021; and countries, cities, authors, journals, and institutions publishing the most papers on tissue spheroids. Scopus database was selected due to its high-quality research coverage. Scopus is a peer-reviewed database of peer-reviewed literature, which contains over 200 million records published in more than 80,000 peer-reviewed scientific publications, including books, chapters, conference papers, and proceedings. Scopus is an abstract and citation database covering the fields of science, technology, medicine, social sciences, and the arts and humanities.

In addition, Scopus includes attractive tools useful for scientometrics, facilitating the performance evaluation of publications, authors, institutions, countries, and regions. It also enables researchers to determine the latest research areas and emerging fields. A scientometric analysis on tissue spheroids was done recently by Rodriguez-Salvador et al.[20] where information from both Scopus and Web of Science was analyzed. However, with the present study being focused on the specific approach of tissue spheroids as building blocks, we considered it to be more convenient to work only with one database, in this case, Scopus, which effectively facilitated the search and the further analysis with the powerful analytical tools available.

2. Methodology
A competitive technology intelligence (CTI) process, with a scientometric analysis, was carried out as described by Rodriguez-Salvador et al.[21], this cyclical process comprised ten main steps: (1) Process planning; (2) primary and secondary source identification; (3) establishment of information collection strategy; (4) information collection; (5) expert validation and adjustments; (6) scientometric analysis; (7) expert validation and adjustments; (8) verification of final results; (9) results delivery; and (10) decision-making.

A scientometric analysis on tissue spheroids in general was done recently by Rodriguez-Salvador et al.[20] The search query applied in that study was considered for this analysis, which gathered all current information regarding tissue spheroids in general, as follows:

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(((spheroid* PRE/1 (cell OR cellular)) OR (“3 d spheroid") OR ((3D OR “three-dimensional” OR “3 dimensional” OR “three d") PRE/1 spheroids)) OR (cancer PRE/1 spheroids) OR (tumour* PRE/1 spheroids) OR (tumorspheres OR tumorspheres OR tumorospheres) OR (cell* PRE/4 spheroid*) OR (multicell* PRE/3 spheroid*) OR (tissue* PRE/2 spheroid*) OR ((hepat* OR liver OR pancreas* OR thyroid OR organotypic OR cardiomyocyte) PRE/0 spheroid) OR (“self assembl* spheroid”) OR cardiosphere OR (cell AND (spheroid PRE/0 (formation OR invasion OR culture))) AND (“tissue engineering” OR “regenerative medicine”) AND NOT (plant OR graphite OR bact* OR alga* OR “solar cell*” OR “eutectic cell*” OR yeast OR spheroidin OR alloy OR rhodopseudomonas OR phytoplankton OR mycobacteria OR larva OR protista OR volvox OR coli OR “non-spheroid*” OR anisotropic OR pollon OR coral OR biofilm OR sponge OR plankton OR microalgae OR OR dictyostelium OR microbial OR microbe OR phytoplankton OR saccharomyces OR eps OR candida OR sea OR food OR amoeba OR “date palm” OR kelvin OR peanut OR lanata OR yew OR roseus OR ajuga OR “protein aggregates” OR antenna OR bater* OR foam OR barnacle OR oblate OR review OR overview))
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Scientific publications were retrieved from the Scopus database. Scopus is an abstract and citation database of peer-reviewed literature, which contains over 5000 publishers and 70 million items across scientific disciplines (1970 to present)[19].

The search and retrieval of scientific publications were performed for journal articles and conference papers.
published between January 1, 2015, and December 31, 2021. The collected publications were first manually classified based on specific applications of tissue spheroids in general. Next, publications describing the use of tissue spheroids as building blocks were selected manually for further analysis.

Collected publications underwent scientometric analysis. First, subject areas were determined based on Scopus subject categorization. The growth kinetics of publication activity within the retrieval period was identified, as were the most prolific countries, cities, journals, and institutions. The most cited articles were determined as well.

3. Results

In total, 777 publications from Scopus were retrieved for the period between January 1, 2015, and December 31, 2021, on tissue spheroids in general. After identifying the publications that focused on tissue spheroids as building blocks, a total of 177 publications were obtained. These publications were assessed in terms of the methods employed, with two principal categories: Seeding and Biofabrication. Publications were included in the Seeding category if they described manual injection of tissue spheroids or a process in which tissue spheroids were poured from one container into another to develop a larger tissue construct. Publications were included in the Biofabrication category if they described the use of a bioassembly or bioprinting technique for the deposition or placement of tissue spheroids. Of the 177 publications obtained, 137 publications (77%) described the use of seeding method, while 40 publications (23%) described the use of biofabrication method; 16 of the biofabrication publications described bioassembly, while 24 described bioprinting. The distribution of scientific publications based on the methods employed to build tissue spheroids used as building blocks is presented in Figure 1.

Based on the classification of science disciplines in the Scopus database, subject areas were identified. In the present study, the analysis of all 177 publications revealed eight main subjects as shown in Figure 2: These subjects include multidisciplinary (60%), biochemistry genetics and molecular biology (18%), medicine (15%); engineering (4%), chemical engineering (1%), material science (1%), pharmacology; toxicology and pharmaceutics (1%), and veterinary (1%). The following fields were included in the multidisciplinary category: Biochemistry; engineering; medicine; material science; chemical engineering; immunology and microbiology; chemistry; pharmacology; toxicology, and pharmaceutics; physics and astronomy; computer science. Regarding publications in which tissue spheroids were seeded, the top five subject areas were multidisciplinary (52%); biochemistry, genetics, and molecular biology (21%); medicine (15%); engineering (4%); and chemical engineering (4%). Regarding publications in which tissue spheroids were deposited or placed by using biofabrication, the top five subject areas were multidisciplinary (62%); biochemistry, genetics and molecular biology (19%); medicine (8%); engineering (8%); and material science (3%).

The growth kinetics of publications on the use of tissue spheroids as building blocks is shown in Figure 3. The retrieval period for this study was January 1, 2015, and December 31, 2021. In terms of overall publications, papers published by year did not exhibit a specific trend; nevertheless, publications regarding tissue spheroids as building blocks showed increasing growth from 21 publications in 2015 to 40 publications in 2021. The Seeding category exhibited an increase from 16 publications in 2015 to 35 publications in 2021. The Biofabrication category remained steady with 5 publications in 2015 and 5 publications in 2021. While the most productive years for the Biofabrication category were 2016 and 2020 (7 publications each), and 2021 (40 publications) for the seeding category.

In addition, the countries and cities with the largest number of publications related to tissue spheroids as building blocks were also determined. These results are presented in Figures 4A, B, 5A, B, and 6A, B.
41 overall publications (18%), United States was the most prolific country. The second and third most prolific countries were China and Japan, with 37 (16%) and 30 (13%) overall publications, respectively. Analysis of the Seeding category showed that United States was the most prolific country with 29 (17%) publications, followed by Japan and China with 25 (15%) each. In Biofabrication category, the United States and China were the two most prolific countries with 12 (21%) publications each, the second most prolific countries were Germany, Japan, and South Korea with 5 (9%) publications each. Regarding the top cities for overall publications, the top three were Beijing, China (13 publications, 4%); Tokyo, Japan (10 publications, 3%); and Guangzhou, China (10 publications, 3%). The analysis of the Seeding category showed that the most prolific city was Beijing, China with 10 publications (5%), followed by Tokyo, Japan with nine publications (4%), and Guangzhou, China with 8 publications (3%). Finally, the analysis of the Biofabrication category showed that the most prolific cities were Seoul, South Korea (3 publications, 4%), Beijing, China (3 publications, 4%); and ten cities with 2 publications each (3%): Cambridge, United States; Cambridge, United Kingdom; Fukuoka, Japan; Guangzhou, China; Hangzhou, China; Heidelberg, Germany; London, United Kingdom; Maastricht, Netherlands; Moscow, Russia; and New York City, United States.

Among all publications in this study, a total of 1304 authors were identified. However, distinguishing the leading authors from other authors could not be achieved in this study since the use of tissue spheroids as building blocks is still a novel technology. Nevertheless, the top-cited Scopus articles in this study are presented in Table 1 (overall as building blocks), Table 2 (building blocks in the Seeding category), and Table 3 (building blocks in the Biofabrication category). The top 6 cited Scopus publications overall belong to the seeding category. The most cited article overall and in the Seeding category, with 119 citations, was *Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related...*

![Figure 3](image)

**Figure 3.** The number of publications on the use of tissue spheroids as building blocks, by year of publication. The straight blue line corresponds to overall publications, dashed purple line corresponds to the seeding category, and dashed yellow line corresponds to the biofabrication category.

![Figure 4](image)

**Figure 4.** Global scientific trends in tissue spheroids as building blocks. A summary of the publications that are indexed in Scopus according to (A) the ten most frequent countries and (B) cities of the authors; (C) the ten journals with the most occurrences of the searched terms; and (D) the ten most frequent organizational affiliations of the authors.
acute-on-chronic liver failure: A randomized controlled trial\cite{22}, where the use of mesenchymal stromal cells (MSCs) to decrease mortality in hepatitis B virus-related acute-on-chronic liver failure was studied. Followed by 3D spheroid culture enhances survival and therapeutic capacities of MSCs injected into ischemic kidney\cite{23}, the second most cited article overall and in the Seeding category with 91 citations. This publication investigates the in vitro therapeutic effects of 3D spheroids of human/adipose MSCs in the treatment of acute kidney injury (AKI). The third most cited article overall, also in the Seeding category, was Combined intramyocardial delivery of human pericytes and cardiac stem cells additively improves the healing of mouse infarcted hearts through stimulation of vascular and muscular repair\cite{24}, with 88 citations. In this article, the use of saphenous vein-derived pericytes and cardiac stem cells as a repair tool in myocardial infarction was analyzed. On the other hand, the top-cited article in the Biofabrication category was Accessible bioprinting: Adaptation of a low-cost 3D-printer for precise cell placement and stem cell differentiation\cite{25}, cited 60 times. The article laid out an accessible 3D bioprinter capable of printing and differentiating human induced pluripotent stem cells (hiPSC) into Gletrex. With 58 citations Towards Single-Step Biofabrication of Organs on a Chip via 3D Printing\cite{26} is the second most cited article. It is a review where organ-on-a-chip engineering applies microfabrication of living tissue with microscale fluid that mimics human organs with 3D printing. The third most cited article was Airflow-Assisted 3D Bioprinting of Human Heterogeneous Microspheroidal Organoids with Microfluidic Nozzle\cite{27}, with 51 citations. The study presents a contemporary airflow-assisted 3D bioprinting method that creates spiral microarchitectures inside the microspheroids to achieve heterogeneous tissue spheroids.
Table 1. Top ten cited Scopus publications related to tissue spheroids as building blocks.

| S. No. | Title                                                                                                                                  | Authors                                                                                                                                | Year | Source                                      | Scopus cites |
|--------|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|------|---------------------------------------------|--------------|
| 1      | Allogeneic bone marrow–derived mesenchymal stromal cells for hepatitis B virus–related acute-on-chronic liver failure: A randomized controlled trial[22] | Lin, BL; Chen, JF; Qiu, WH; Wang, KW; Xie, D; Chen, XY; Liu, QL; Peng, L; Li, JG; Mei, YY; Weng, WZ; Peng, YW; Cao, HJ; Xie, JQ; Xie, SB; Xiang, AP; Gao, ZL. | 2017 | Hepatology (Baltimore, Md.) 66 (1), 209-219 | 119          |
| 2      | 3D spheroid culture enhances survival and therapeutic capacities of MSCs injected into ischemic kidney[23]                                  | Xu, Y; Shi, T; Xu, A; Zhang, L.                                                                                                           | 2016 | Journal of Cellular and Molecular Medicine 20 (7), 1203-1213 | 91           |
| 3      | Combined intramyocardial delivery of human pericytes and cardiac stem cells additively improves the healing of mouse infarcted hearts through stimulation of vascular and muscular repair[24] | Avolio, E; Meloni, M; Spencer, H; Riu, F; Katre, R; Mangialardi, G; Oikawa, A; Rodriguez-Arabaozaza, I; Dang, Z; Mitchell, K; Reni, C; Alvino, V; Rowlinson, J; Livi, U; Cesselli, D; Angelini, G; Emanuelli, C; Beltrami, A; Madeddu, P. | 2015 | Circulation Research 116 (10), e81-94        | 88           |
| 4      | Transplantation of a human iPSC-derived hepatocyte sheet increases survival in mice with acute liver failure[25]                           | Nagamoto, Y; Takayama, K; Ohashi, K; Okamoto, R; Sakurai, F; Tachibana, M; Kawabata, K; Mizuguchi, H.                                  | 2016 | Journal of Hepatology 64 (5), 1068-1075     | 80           |
| 5      | Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels[25]                              | Jha, AK; Tharp, KM; Ye, J; Santiago-Ortiz, JL; Jackson, WM; Stahl, A; Schaffer, DV; Yeghiazarians, Y; Healy, KE.                      | 2015 | Biomaterials 47, 1-12                        | 77           |
| 6      | Three-dimensional testicular organoid: A novel tool for the study of human spermatogenesis and gonadotoxicity in vitro[26]              | Pendergraft, SS; Sadri-Ardekani, H, Atala, A; Bishop, CE.                                                                              | 2017 | Biology of Reproduction 96 (3), 720–732     | 67           |
| 7      | Accessible bioprinting: Adaptation of a low-cost 3D-printer for precise cell placement and stem cell differentiation[27]               | Reid, JA; Mollica, PA; Johnson, GD; Ogle, RC; Bruno, RD; Sachs, PC.                                                                     | 2016 | Biofabrication 8 (2), 025017               | 60           |
| 8      | Microfluidic Templated Multicompartment Microgelss for 3D Encapsulation and Pairing of Single Cells[28]                                 | Zhang, L; Chen, K; Zhang, H; Pang, B; Choi, CH; Mao, AS; Liao, H; Utech, S; Mooney, DJ; Wang, H; Weitz, DA.                           | 2018 | Small 14 (9)                               | 60           |
| 9      | Towards Single-Step Biofabrication of Organs on a Chip via 3D Printing[29]                                                            | Knowlton, S; Yenilmez, B; Tasoglu, S.                                                                                                 | 2016 | Trends in Biotechnology 34 (9), 685-688    | 58           |
| 10     | Airflow-Assisted 3D Bioprinting of Human Heterogeneous Microspheroideal Organoids with Microfluidic Nozzle[30]                          | Zhao, H; Chen, Y; Shao, L; Xie, M; Nie, J; Qiu, J; Zhao, P; Ramezani, H; Fu, J; Ouyang, H; He, Y.                                     | 2018 | Small 14 (39)                              | 51           |
Table 2. Top ten cited Scopus publications related to tissue spheroids as building blocks in the Seeding category.

| S. No. | Title                                                                 | Authors                                                                                           | Year | Source                                                                                         | Scopus Cites |
|-------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------------------|--------------|
| 1     | Allogeneic bone marrow–derived mesenchymal stromal cells for hepatitis B virus–related acute-on-chronic liver failure: A randomized controlled trial\(^{[22]}\) | Lin, BL; Chen, JF; Qiu, WH; Wang, KW; Xie, D; Chen, XY; Liu, QL; Peng, L; Li, JG; Mei, YY; Weng, WZ; Peng, YW; Cao, HJ; Xie, JQ; Xie, SB; Xiang, AP; Gao, ZL. | 2017 | Hepatology (Baltimore, Md.) 66 (1), 209-219                                                    | 119          |
| 2     | 3D spheroid culture enhances survival and therapeutic capacities of MSCs injected into ischemic kidney\(^{[23]}\) | Xu, Y; Shi, T; Xu, A; Zhang, L.                                                                     | 2016 | Journal of Cellular and Molecular Medicine 20 (7), 1203-1213                                   | 91           |
| 3     | Combined intramyocardial delivery of human pericytes and cardiac stem cells additively improves the healing of mouse infarcted hearts through stimulation of vascular and muscular repair\(^{[24]}\) | Avolio, E; Meloni, M; Spencer, H; Riu, F; Katare, R; Mangialardi, G; Oikawa, A; Rodriguez-Arabaolaza, I; Dang, Z; Mitchell, K; Reni, C; Alvino, V; Rowlinson, J; Livi, U; Cesselli, D; Angelini, G; Emanueli, C; Beltrami, A; Madeddu, P. | 2015 | Circulation Research 116 (10), e81-94                                                        | 88           |
| 4     | Transplantation of a human iPSC-derived hepatocyte sheet increases survival in mice with acute liver failure\(^{[31]}\) | Nagamoto, Y; Takayama, K; Ohashi, K; Okamoto, R; Sakurai, F; Tachibana, M; Kawabata, K; Mizuguchi, H. | 2016 | Journal of Hepatology 64 (5), 1068-1075                                                    | 80           |
| 5     | Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels\(^{[32]}\) | Jha, AK; Tharp, KM; Ye, J; Santiago-Ortiz, JL; Jackson, WM; Stahl, A; Schaffer, DV; Yeghiazarians, Y; Healy, KE. | 2015 | Biomaterials 47, 1-12                                                                         | 77           |
| 6     | Three-dimensional testicular organoid: A novel tool for the study of human spermatogenesis and gonadotoxicity in vitro\(^{[33]}\) | Pendergraft, SS; Sadri-Ardekani, H, Atala, A; Bishop, CE.                                             | 2017 | Biology of Reproduction 96 (3), 720–732                                                     | 67           |
| 7     | Microfluidic Templated Multicompartment Microgels for 3D Encapsulation and Pairing of Single Cells\(^{[34]}\) | Zhang, L; Chen, K; Zhang, H; Pang, B; Choi, CH; Mao, AS; Liao, H; Utech, S; Mooney, DJ; Wang, H; Weitz, DA. | 2018 | Small 14 (9), 60                                                                            | 60           |
| 8     | Three-dimensional liver-derived extracellular matrix hydrogel promotes liver organoid’s function\(^{[35]}\) | Saheli, M; Sepantafar, M; Pournasr, B; Farzaneh, Z; Vosough, M; Piryaie, A; Baharvand, H.           | 2018 | Journal of Cellular Biochemistry 119 (6), 4320-4333                                           | 47           |
| 9     | One step fabrication of hydrogel microcapsules with hollow core for assembly and cultivation of hepatocyte spheroids\(^{[36]}\) | Siltanen, C; Diakatou, M; Lowen, J; Haque, A; Rahimian, A; Stybayeva, G; Revzin, A.               | 2017 | Acta Biomateriala 1, 428-436                                                                  | 47           |
| 10    | Improved physiological properties of gravity-enforced reassembled rat and human pancreatic pseudo-islets\(^{[37]}\) | Zuellig, RA; Cavallari, G; Gerber, P; Tschopp, O; Spinas, GA; Moritz, W; Leumann, R.               | 2017 | Journal of Tissue Engineering and Regenerative Medicine 11 (1), 109-120                     | 32           |
The most prolific journals with publications on the use of tissue spheroids as building blocks were detected (Figures 4C and 5C). For overall publications, Biofabrication was the most prolific journal with 6 publications (3%), followed by Trends in Biotechnology with 5 publications (3%) each. The six most prolific journals in the Seeding category were the Cells, Chinese Journal of Tissue...
Engineering Research; Journal of Bioscience and Bioengineering; Methods in Molecular Biology; Scientific Reports; and Stem Cell and Therapy with 4 publications (3%) each. In the Biofabrication category, the most prolific journal was Biofabrication with 4 publications (10%), followed by Progress in Biomedical Optics and Imaging - Proceedings of SPIE with 2 publications (5%).

In Figures 4D and 5D, the top institutions with the largest number of publications on the use of tissue spheroids as building blocks are shown. Overall, 324 institutions were identified worldwide. The University of Tokyo (Tokyo, Japan) was the most prolific institution with 7 publications (2%). The second most prolific institution was Osaka University (Osaka, Japan) with 6 publications (1%), and the third most prolific institutions were Kyushu University (Fukuoka, Japan) and Harvard University (Cambridge, United States) with 5 publications (1%). In the Seeding category, the most prolific institution was the University of Tokyo (Tokyo, Japan) with 6 publications (2%), followed by Osaka University (Osaka, Japan) with 5 publications each (2%). Harvard University (Cambridge, United States), North Carolina State University (Raleigh, United States), University of California (San Diego, United States), and University of North Carolina (Chapel Hill, United States) were tied up as the third most prolific institutions with 4 publications (1%) each. In the Biofabrication category, the seven most prolific institutions were the Jinan University (Guangzhou, China), Kyushu University (Fukuoka, Japan), Maastricht University (Maastricht, Netherlands), Shanghai University (Shanghai, China), Sungkyunkwan University (Seoul, South Korea), Tsinghua University (Beijing, China), and Zhejiang University (Hangzhou, China) with 2 publications (2%) each. In this category, the remaining affiliations had 1 publication each.

In addition to the scientometric analysis, in this study the following were identified: techniques used, types of cells, culture time, and applications of tissue spheroids as building blocks. Tables 4 and 5 present this kind of detail regarding the most representative papers in both the Seeding and Biofabrication categories. Similar to the previous research of tissue spheroids in general by Rodriguez-Salvador et al.[20] results obtained in this analysis show that there is a variety of cells used in tissue spheroids as building blocks. Specifically, the predominant type of cells for the Seeding category are spermatogonial stem cells (SSCs)[33], mesenchymal stem cells (MSCs)[22,23], human umbilical vein endothelial cells (HUVECs)[34], and for the Biofabrication category, hiPSC[25] L929 cells[27] and adipose-derived mesenchymal cells (ADSC)[49]. Furthermore, different types of 3D bioprinting techniques are used depending on the goal intended. Similarly, no optimal size or arrangement of the spheroids has been defined, but advances detected in this analysis show that the spheroid diameter can be from 200 µm to 500 µm. Whereas culture time ranges from 2 to 7 days. Results also exhibit an extensive application of tissue spheroids as building blocks, which comprise cell biology, cell therapy, and tissue engineering for regenerative medicine and organ modeling. As an example, the use of tissue spheroids in predictive first tier drug-screening, as models of human testicular function, and in cell therapy for AKI were identified. New 3D bioprinting systems have also been developed to provide superior performance on tissue spheroids fabrication.

4. Discussion

Research on the use of tissue spheroids as building blocks is growing moderately, covering eight subject areas: Multidisciplinary; Biochemistry Genetics and Molecular Biology; Medicine; Engineering; Chemical Engineering; Material Science; Pharmacology, Toxicology, and Pharmaceutics; and Veterinary. More than half of the analyzed publications presented interest in multiple topics. This trend may be due to the need to design new tissue spheroid models and novel technologies that enable the generation of diverse tissue constructs[5].

The results show that the level of scientific production regarding the use of tissue spheroids as building blocks is at an early stage and that there is a small number of publications available, in total 177 from January 1, 2015, to December 31, 2021. However, scientific production is expected to grow in the future, as a continuous increase in the number of publications in the field was observed from 2015 to 2021 (21 – 40 overall and 16 – 35 in the Seeding category). On the other hand, the Biofabrication category remained almost steady (5 publications in both years). Biofabrication is a novel technology whose main long-term goal is to produce functional tissue and organoids for transplantation. Results obtained showed that biofabrication research is mainly focused on cosmetic and drug testing, tissue regeneration, and medical[29].

The most prolific countries overall and in the Biofabrication category were the United States and China. While in the Seeding category, the most prolific countries were the United States, China, and Japan. The United States, China, and Japan have the largest investments in research and development. Each country invests 476, 346, and 170 billion purchasing power parity (SPPP) per year, respectively, with more than 70% originating in the business sector[29]. Notably, SPPP is a currency exchange rate used to standardize the global purchasing power of various currencies[30].

Furthermore, the most prolific cities and institutions, in terms of publications on the use of tissue spheroids as building blocks overall and the Seeding category, were concentrated in China and Japan. While in the Biofabrication category, the most prolific cities and
Institutions were concentrated in South Korea and China. According to the Alliance of Regenerative Medicine[38], in the last years, the number of developers in Asia-Pacific has increased by 119%.

In this research, some relevant characteristics involved in the production of tissue spheroids as building blocks that belong to both the Seeding and Biofabrication categories were identified in the publications. In addition, an analysis of the ten most cited Scopus publications was done. Insights obtained show the use of different techniques, such as hanging drop culture[22], suspension culture[24, 48], spheroid-laden hydrogels[32, 34], 96 well format hanging drop plate[33], microextrusion based bioprinting[25], and air-flow assisted 3D bioprinting[27]. Furthermore, the predominant types of cells used were MSCs[22, 23, 34], cardiac stem cells[24], hiPSC[23, 31], SSC[33], and HUVECs[34]. Finally, tissue spheroids were mostly applied to enhance acute kidney injury recovery[22, 23, 31] and treat infarcted hearts[24].

Novel technological solutions have been designed for the specific use of tissue spheroids as building blocks through biofabrication methods. Examples of those are the aforementioned Kenzan with the Regenova bio-3D-printer, and the FABION extrusion-based bioprinter[39, 40]. Using tissue spheroid characteristics, these customized systems allow the creation of tissue construct designs that may facilitate the evolution of the health industry.

### Table 4. Global trend: tissue spheroids as building blocks applying seeding techniques.

| Article | Authors | Year/source | Impact Analysis |
|---------|---------|-------------|-----------------|
| Three-dimensional testicular organoid: A novel tool for the study of human spermatogenesis and gonadotoxicity in vitro[33] | Pendergraft, SS; Sadri-Ardekani, H; Atala, A; Bishop, CE | 2017/Biology of Reproduction | “The overall goal of this study was to develop a 3D in vitro human testis organoid culture system for use as both a predictive first tier drug-screening tool and as a model of human testicular function.” In this research spermatogonial stem cell, Leydig cells, and sertoli cells were seeded into a 96 well format hanging drop plate in a concentration of 10,000 cells/40 µL and cultured for 48.0 h |
| 3D spheroid culture enhances survival and therapeutic capacities of MSCs injected into ischemic kidney[23] | Xu, Y; Shi, T; Xu, A; Zhang, L. | 2016/Journal of Cellular and Molecular Medicine | “In this study, we aimed to investigate the therapeutic effects of 3D spheroids of human adipose-derived MSCs for acute kidney injury (AKI). […] 3D spheroids of mesenchymal stem cells (MSCs) produced higher levels of ECM proteins […] and exhibited stronger anti-apoptotic and anti-oxidative capacities under ROS stimulation in vitro. These results implied that 3D spheroids would have improved survival than 2D cultured cells in vivo.” Around 25,000 cells/35 µL were placed employing the hanging drop culture technique. The spheroids were cultured for 72.0 h at 37.0°C with 5.0% CO₂. As a result, 3D spheroids with superior performance were developed, they had a size of approximately 200 µm |
| Microfluidic Templated Multicompartment Microgels for 3D Encapsulation and Pairing of Single Cells[34] | Zhang, L; Chen, K; Zhang, H; Pang, B; Choi, CH; Mao, AS; Liao, H; Utech, S; Mooney, DJ; Wang, H; Weitz, DA. | 2018/Small | A novel method that develops monodisperse multicompartment hydrogels for the growth of tissue spheroids was created. “Controlled encapsulation and pairing of single cells within a confined 3D matrix can enable the replication of the highly ordered cellular structure of human tissues. Microgels with independently controlled compartments that can encapsulate cells within separately confined hydrogel matrices would provide precise control over the route of pairing single cells.” A viability assessment was carried out with stem cells and human umbilical vein endothelial cells (HUVECs) were used in two concentrations, 2,500 and 3,500 cells/µL. “This microfluidic technique represents a significant step toward high-throughput single cells encapsulation […] significant importance for cell biology, cell therapy, and tissue engineering.” |
Table 5. Global trend: tissue spheroids as building blocks applying biofabrication techniques.

| Article | Authors | Year/Source | Impact Analysis |
|---------|---------|-------------|-----------------|
| Accessible bioprinting: Adaptation of a low-cost 3D-printer for precise cell placement and stem cell differentiation \(^{[25]}\) | Reid, JA; Mollica, PA; Johnson, GD; Ogle, RC; Bruno, RD; Sachs, PC. | 2016/Biofabrication | To the best of the author’s knowledge, their system is the first “3D printed, bioprinting system” to reliably achieve single-cell print resolutions within 50.0 µm resolution while also exerting minimal unwanted impact on the cells viability and post-printing fate. The plotting routine was set to dispense a number of 100 nL droplets 200.0 µm (X, Y) apart and 250.0 µm (Z) from the plate bottom into wells of a 96 well plate containing 50.0 µL of a 1:1 mixture of growth factor reduced Geltrex and differentiation supportive media.” The human induced pluripotent stem cells (hiPSC) “aggregates were incubated at 37.0°C, 5.0%CO₂ for 7 days. […] an accessible open-source 3D bioprinter capable of serving the needs of any laboratory interested in 3D cellular interactions and tissue engineering.” |
| Airflow-Assisted 3D Bioprinting of Human Heterogeneous Microspheroidal Organoids with Microfluidic Nozzle \(^{[27]}\) | Zhao, H; Chen, Y; Shao, L; Xie, M; Nie, J; Qiu, J; Zhao, P; Ramezani, H; Fu, J; Ouyang, H; He, Y. | 2018/Small | “[…] heterogeneous microspheroids are drawing much attention as a promising tool to carry multiple cell types in separated phases […] a novel airflow-assisted 3D bioprinting method is reported, which can print versatile spiral microarchitectures inside the microspheroids.” Printed spheroids presented a 0.5 mm diameter with an inner spiral of 10.0 µm. L929 cells were cultured for 4 days and had a 90.0% viability |
| Magnetic nanoparticle loaded human adipose derived mesenchymal cells spheroids in levitated culture \(^{[49]}\) | Labusca, L; Herea, DD; Minuti, AE; Stavila, C; Danceanu, C; Grigoras, M; Ababei, G; Chiriac, H; Lupu, N. | 2021/Journal of Biomedical Materials Research Part B: Applied Biomaterials | In this study, the authors used magnetic levitation to “obtain highly reproducible human ADSC–MNP assembled in 3D culture (spheroids) with the purpose of increasing stem cell specific properties. […] Levitated spheroid culture of ADSC-MNP can be further tested for various application in regenerative medicine and organ modeling.” Adipose-derived mesenchymal cells (ADSC) spheroids were combined with magnetic nanoparticles (MNP) and cultured in a 96 well plates with a concentration of 1000 cells at 37.5°C for 7 days. “For magnetic field (MF) stimulation and establishment of levitated culture, permanent NdFeB magnets with diameter of 8 mm each were placed on the top of the 96 well plate and incubated.” |

5. Conclusion

A scientometric analysis as part of a CTI process was carried out to determine the scientific research dynamics of tissue spheroids as building blocks. To accomplish this, scientific publications were retrieved from Scopus for the period between January 1, 2015, and December 31, 2021, then organized and analyzed. The results revealed that the application of tissue spheroids as building blocks is still at an early stage. During our research, publications were classified into two fundamental categories based on the techniques used to deposit or assemble tissue spheroids as building blocks: Seeding and Biofabrication. At present, manual seeding predominates over the automated process of biofabrication, however, with the arrival of digitalization, it is expected that new technologies such as artificial intelligence and robotics will be incorporated in the future, providing more robust processes and tissues with superior performance. To describe the overall research publications landscape and specific trends for seeding and biofabrication methods, the subject area distributions, the most prolific countries, cities, journals, and institutions, and the most cited articles regarding this
topic were identified. In the Overall Publications, Seeding, and Biofabrication categories, the Multidisciplinary subject predominated. On the other hand, while in the Overall Publications and Seeding category the number of publications increased, in the Biofabrication category it remained steady. In terms of countries, the United States, China and Japan predominated. The most cited publications overall belonged to the Seeding category. Regarding journals in the Overall Publications and Biofabrication categories, biofabrication was the top journal, while in the Seeding category there were six most prolific journals. Insights obtained also show that in the overall publications and seeding categories, the University of Tokyo ranked top considering number of publications in the field, while in the Biofabrication category, the seven most prolific institutions were identified predominantly from China.

Insights obtained may add value for stakeholders in academia, industry, and the government, who are interested in the incorporation of innovative health technologies in the decision-making process, by enabling them to keep abreast of current global trends. In addition, these results may be applied as a tool to address research efforts in the tissue engineering and regenerative medicine fields to create novel technologies that improve the current health industry.

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Conflict of interest
The authors declare no conflicts of interest.

Author contributions
M.R-S. conceived the idea and contributed to the paper by guiding its organization, content, and further corrections according to the reviewers. I.F-M developed the data gathering, the Scientometric analysis, and drafted the manuscript by adjusting the first approach, complementing it and presenting the analytics before and after reviewers evaluation. B. E. P-B developed the first manuscript general approach. J.R. L-R presented the first theoretical part.

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