PATENTS

3D and 4D bioprinted human model patenting and the future of drug development

Bioprinted 3D and 4D tissues and organs are expected to revolutionize the biomedical field, eliminating the need for laboratory animals, but little is known about the future impacts of these technologies on drug development.

In medical and biomedical research, animals such as rodents, rabbits and dogs are used for the development of new drugs, vaccines, biologics, devices and medical procedures[1,2]. The use of animals for scientific purposes is normally regulated by ethical guidelines, country-specific legislation and international legislation aimed at protecting their rights and welfare[2–4]. There are many reasons behind this system: animals may be considered good models for humans and human diseases[3]; they may be an established research approach for the study of the mechanisms of disease progression[5]; legal and regulatory requirements may demand in vivo preclinical safety testing before a candidate drug can be tested on humans[6,7]; models that mimic the physiological conditions of human organs may be lacking[8]; alternative methods may not have been implemented[7]; and there may be research-culture-related factors[9].

Although animal models are standard in preclinical studies, the high failure rates of drugs in clinical trials suggest that interspecies differences make animals neither good models of humans disease nor reliable predictors of toxicity[5,10,11]. This has given rise to growing ethical, political and social pressures to develop alternatives to the use of animals in research[10].

In light of these issues, some technologies are being developed to be used as alternatives to animal models. They include in chemico models (test tubes that simulate reactions in the organism)[12], in silico models (ones based on computer modeling)[13], and in vitro models (including two- and three-dimensional (3D) cell cultures)[14]. As they still lack scientific validation, these methods are used along with animal models[6]. Among them, in vitro models — 3D models in particular — have started to gain in importance, since they are considered the most promising for mimicking in vivo models[15].

Three-dimensional in vitro models can be seeded with multiple different techniques, such as scaffold-based and scaffold-free methods[15]. They can also be bioprinted, which allows more precise control over the cell microenvironment[16]. Bioprinting is a process that makes use of cells, biomaterials, biomaterial scaffolds, growth factors and other biological factors[17–19] for printing biological and functional systems[19]. Bioprinting is also known as biofabrication, 3D bioprinting and four-dimensional (4D) bioprinting[20–22]. In 3D bioprinting, living cells are deposited layer by layer in a biocompatible hydrogel, giving the model the desired structure[23]. In 4D bioprinting, the materials, besides being living cells, are also responsive to stimuli[22]. Hereafter, we will use 3D/4D bioprinting to refer to either or both.

Bioprinted 3D/4D tissues and organs are expected to revolutionize the biomedical field and may represent a new research avenue for drug testing[24,25], potentially reducing costs[26] and increasing the speed of drug development[27] and success rates of drug candidates[26]. Some bioprinted models have already proved effective for toxicity

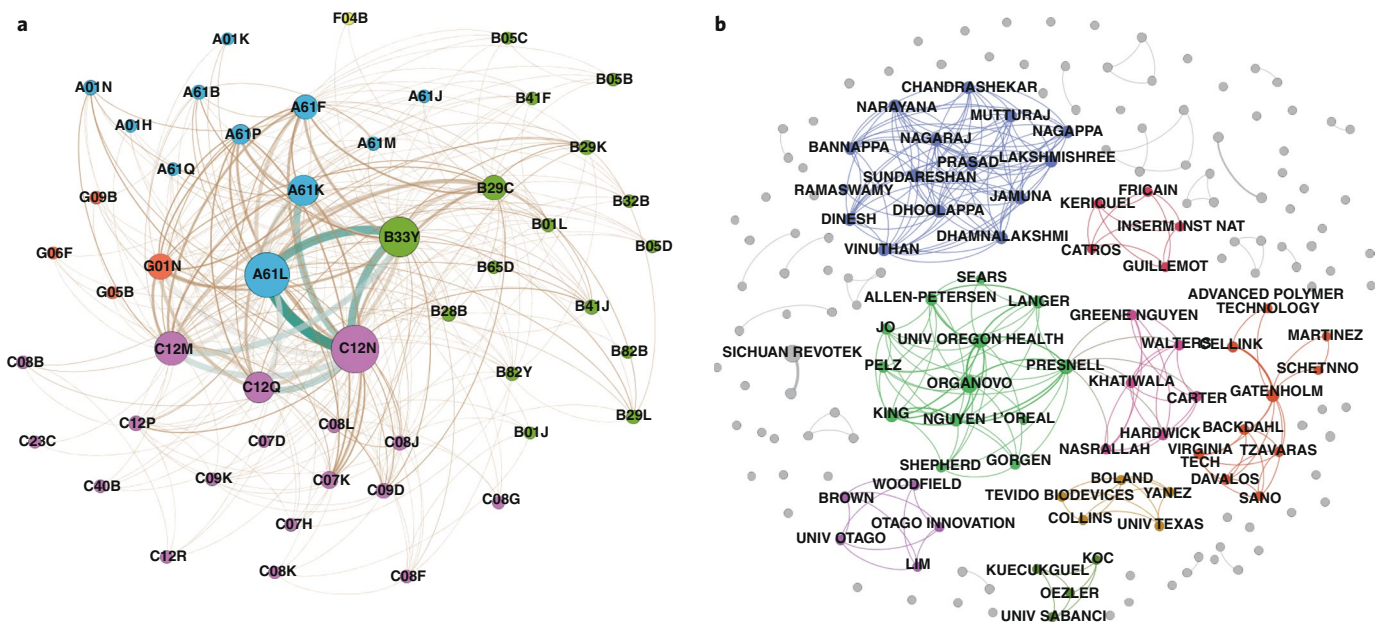

Fig. 1 | Networks of technology areas and patent assignees of bioprinting-related patents. a, Technology areas. **b**, Patent assignees.

Fig. 2 | Groups of technologies, uses, and applications.

and drug screening, and early tests indicate that they could serve as an alternative to animals and perhaps replace them[28]. However, these models are still under development, requiring scientific validation and repeatability before they can be fully adopted by researchers[29].

At the same time, 3D/4D bioprinted models may not constitute a feasible alternative to animals if whole integrated organisms are needed for drug testing[14] and/or required by law[6,7]. Thus, it is still unknown what implications 3D/4D bioprinted models will have for the use of laboratory animals and the drug development process — that is, discovery and development, preclinical research and clinical research taken as a whole.

Only a few studies have attempted to anticipate the future outcomes of 3D/4D bioprinted models[22,23,30,31], and none have focused on their implications for the drug development process and the use of laboratory animals. Here, we address this gap by analyzing the content of 176 bioprinting-related patents indexed in the Derwent Innovations Index and assessing the opinions of 1,472 researchers from all over the world. The researchers invited to participate in this survey are authors of recent scientific publications related to engineered tissues or organs, drug development and laboratory animals indexed in the Web of Science Core Collection (Supplementary Methods).

Results

Patents related to bioprinting. The 176 bioprinting-related patents analyzed refer to basic patents, meaning new inventions indexed in the Derwent Innovations Index. The patents were filed between 2006 and July 2019, 71% of them between 2016 and 2018. Jointly accounting for 90.9% of the patents, the US (85 patents), China (50), and Canada (25) were the three main priority countries (where the first patent applications are filed).

Figure 1 depicts the networks of (**a**) technology areas (IPC 4-digit codes) and (**b**) patent assignees (an inventor or organization that holds the property right of a patent). In both networks, the size of the nodes reflects their degree (the number of connections a node has). In the network of technology areas, there are 50 nodes (IPC 4-digit codes), 352 edges (number of connections among nodes), and 100% of connected nodes. All nodes were labeled and their colors reflect the IPC group codes. The main co-occurrences are between C12N (microorganisms, enzymes, genetic engineering, etc.) and A61L (related to human necessities), and between A61L and B33Y (additive manufacturing). Considering all 176 patents going down the hierarchy of the IPC scheme (IPC 8-digit), the most frequent codes are the A61L 27/38 (39.2%) and the C12N 5/071 (31.2%). The former refers to 'animal cells (for use in artificial skin A61L 27/60)' and the latter to 'vertebrate cells or tissues, for example, human cells or tissues.' The IPC scheme can be found on the WIPO website (wipo.int/portal/en/).

In the network of patent assignees, there are 173 nodes (patent assignees), 426 edges (which in this case reflects collaboration among patent assignees), and 52.6% of connected nodes. Using the Modularity Class algorithm, we decompose the network in sub-networks (or communities) and identified 92 communities. Only the communities with more than 2% of total nodes were colored (except for Sichuan Revotek, labeled due to its share of patents). Sichuan Revotek is the company with the higher number of patents (15.9% of total patents). Despite that, it collaborates only with Revotek. Together, these two

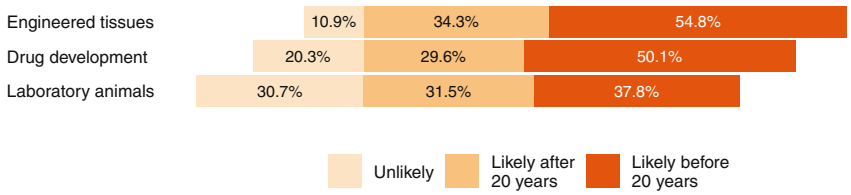

Fig. 3 | Likelihood of 3D/4D bioprinted human tissues/organs replacing the use of laboratory animals in drug development.

assignees are developing inventions related to biobricks, bioprinting devices, bioprinted microvascular constructs (most of their common 5 patents are classified as B29C and B33Y). Organovo ranks second in number of patents (9.7% of total patents) and is the main node of the green community (13 nodes), whose inventions relate to bioprinted models of tumors, liver disorders, skin tissues. Most patents of the green community are classified as C12N, G01N, and A61L.

Figure 2 shows the groups of technologies, uses, and applications into which the 176 patents were classified. Of the eight groups of technologies, 3D tissues and/or organs (hereafter, tissues/organs) and 3D disease models were given priority, as these are technologies involving inventions most closely related to the focus area of this study. Organovo (12 patents) and Sichuan Revotek (4 patents) were the two main organizations patenting inventions for 3D tissues/organs. Accounting for 28.4% of all the patents retrieved, 3D tissues/organs was the largest group of technologies. Its 50 patents were classified into five groups of technology uses, with artificial tissues/organs (21 patents; 41%) and bioprinting of cells/tissues/organs (16 patents; 31%) constituting the two most frequently claimed uses. The inventions classified as artificial tissues/organs were the richest in industrial or research applications. The 21 patents from this group were classified into 14 technology applications, of which drug testing (10 patents), disease model research (9), and toxicity testing (8) were the most frequently occurring application claims.

As for the group of technologies classified as 3D disease models, six of the seven inventions were also classified under 'artificial tissues/organs' for their use. Disease model research and drug screening, with four patents each, were the most frequently claimed industrial or research applications here. The University of Oregon Health Sciences Center (4 patents) and Organovo (3) were the main assignee organizations patenting inventions in 3D disease models.

Survey of researchers. A total of 2,363 researchers participated in this study, 37.7% of which reported having no knowledge of 3D/4D bioprinting applications in the drug development process thus disqualifying them from the survey (Supplementary Table 1). First, the respondents were asked to report on the likelihood of 3D/4D bioprinted human tissues/organs replacing the use of laboratory animals in the drug development process (Fig. 3). This was a mandatory question to which the respondents could choose one of three options: 'likely before 20 years,' 'likely after 20 years,' and 'unlikely.' Those who chose 'unlikely' followed a different route through the questionnaire so that their answers could be compared with the opinions of the researchers who claimed to believe that 3D/4D bioprinted human tissues/organs would at some point replace laboratory animals in drug development. From now on, the terms 'believer' and 'non-believer' are used to simplify the description of the results.

The views of the engineered tissues/organs and drug development respondents were similar (Fig. 3). In both groups, just over 50% believed 3D/4D bioprinted human constructs would likely replace laboratory animals within 20 years. Meanwhile, 34.3% of the engineered tissues/organs respondents and 31.5% of the laboratory animals respondents felt such a transition would only occur in over 20 years' time. The laboratory animals respondents showed a more divergent pattern of responses, with approximately one third of them choosing each of the three options.

Figure 4 shows the views of the researchers who reported they believed that 3D/4D bioprinted human tissues/organs would replace laboratory animals. Since they expected this to happen, they were asked to give their views on the future impacts of these technologies on the development of new drugs. In all three groups, most of the respondents believed these bioprinted constructs would likely: become mainstream in the drug development process; give rise to a new drug development paradigm; radically reduce the problem of adverse reactions to drugs; and radically reduce the need for clinical trials. This last statement was the one with the highest percentages of 'unlikely' responses.

Figures 5 and 6 compare the answers of the respondents who believed and did not believe that 3D/4D bioprinted human constructs would replace laboratory animals. Figure 5 shows their views on five statements related to the future of these technologies in the drug development process. The 'non-believers' group had a higher percentage of 'unlikely' responses than the 'believer' group. For instance, most of the non-believers reported that 3D/4D bioprinted human constructs would be unlikely to accurately predict toxicity in humans, while most believers expressed the opposite view. Overall, most of the non-believer and believer respondents from all three areas of expertise considered all the other statements to be likely within 20 years.

Finally, the respondents were asked to report on the relevance of five challenges that might hamper future uses of 3D/4D bioprinted human constructs in the drug

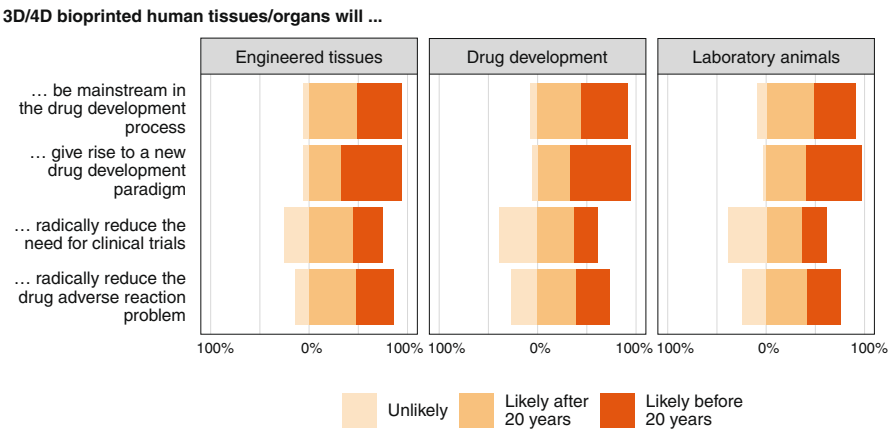

Fig. 4 | Future implications of 3D/4D bioprinted human tissues/organs for the drug development process.

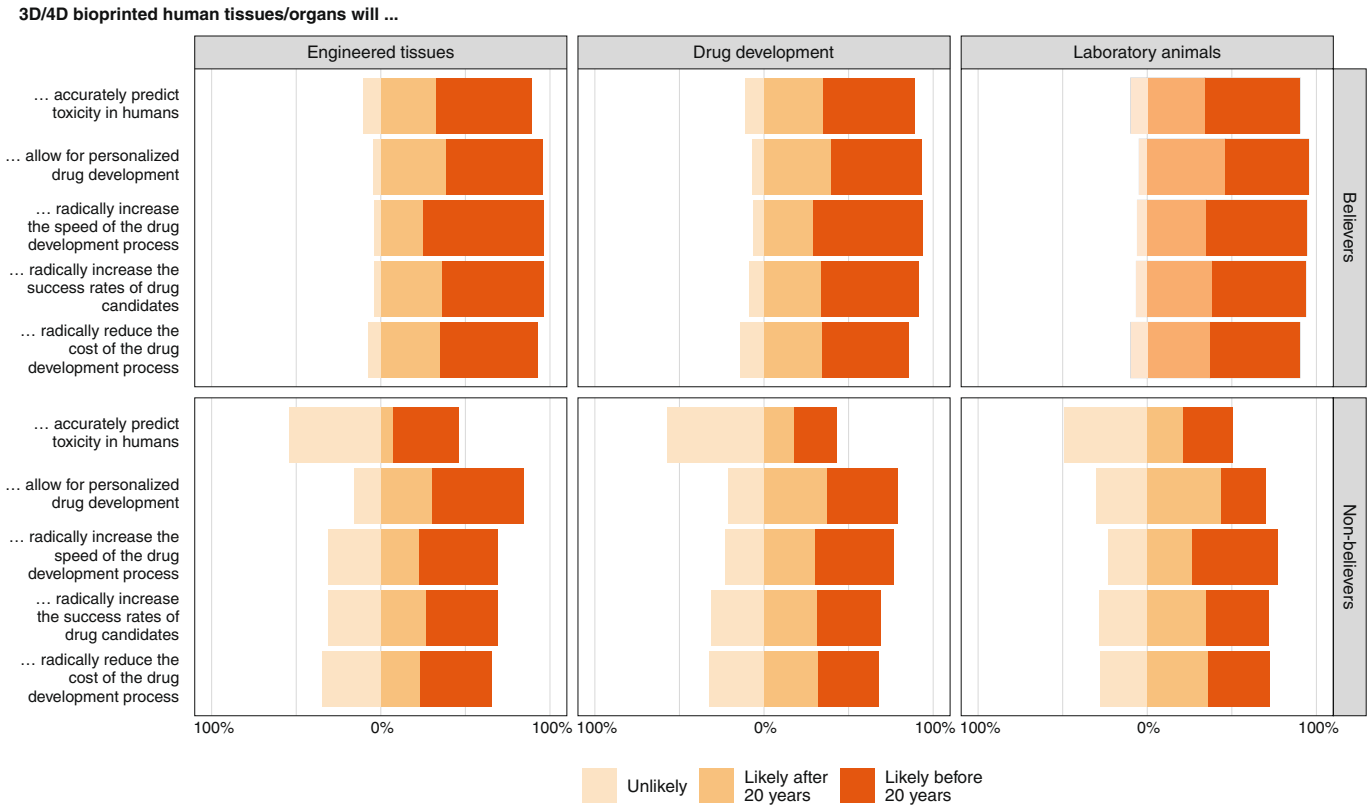

Fig. 5 | Future implications of 3D/4D bioprinted human tissues/organs for the drug development process.

development process (Fig. 6). The views of the engineered tissues/organs, drug development and laboratory animals respondents who believed that 3D/4D bioprinted human constructs would replace laboratory animals were similar overall, and the same applies to the non-believers. However, there were significant differences in the views of the 'believer' and 'non-believer' respondents, especially with regard to the need for whole organism models and ethical challenges. Most non-believers considered the need for whole organism models to be a very important challenge (69.2% to 70.4%), while a much smaller percentage of believers shared this view (29.8% to 35%). As for ethical challenges, the percentage distribution of answers along the scale of importance was more equal among the believers. Meanwhile, the views differed significantly among the non-believers. The scientific community's resistance to the adoption of new technologies was considered only a minor challenge by most non-believers, but a moderate to important challenge by a large share of the believers. Regulatory challenges and lack of scientific validation were considered important or very important by most researchers from both groups.

Discussion

Our results resemble the reviewed literature, suggesting that 3D/4D bioprinting is emerging as a promising method for the production of human tissues and organs, potentially putting an end to the use of laboratory animals. It could even represent a paradigm shift in drug development. Given such expectations, it is of concern that 37.7% of the researchers who agreed to take part in this study were not knowledgeable about the applications of 3D/4D bioprinting to drug development. Since searching for alternatives to animal models is a legal and regulatory requirement[32,33], this lack of knowledge is even more striking given that many of these researchers likely use animals in their research.

It is to be expected that researchers may have some degree of resistance to the substitution of animal models in drug development[34]. This is in part because the use of animals has been the mainstream method for such a long time[35]. Even so, considering the groups together, it is worth noting that most of the respondents expected these bioprinted constructs to replace laboratory animals at some time in the future.

Another possible implication of using 3D/4D bioprinted human tissues/organs in drug development is a reduced need for human clinical trials. If bioprinted models can be developed that are good predictors of toxicity in humans, preclinical tests could be the final phase of drug development[26]. A reduced need for clinical trials thanks to 3D/4D bioprinted human tissues/organs was the proposition that was considered least likely by the respondents who believed that 3D/4D bioprinted human tissues/organs would one day lead to the replacement of laboratory animals. Nevertheless, about 60% of them agreed that over time these bioprinted constructs would radically reduce the need for human clinical trials. All of these respondents also expressed the belief that these models would succeed in predicting toxicity in humans. Toxicity testing was also one of the most frequent applications claimed by the patent assignees.

As is known, adverse reactions to drugs not only undermine patients' quality of life[36], but are also an important source of loss for the pharmaceutical industry due to post-market withdrawals[37]. In our patent analysis, we found large pharmaceutical and cosmetics companies like Novartis and L'Oréal investing in the development of bioprinted models. Bioprinters enable tissues to be printed using cells from different

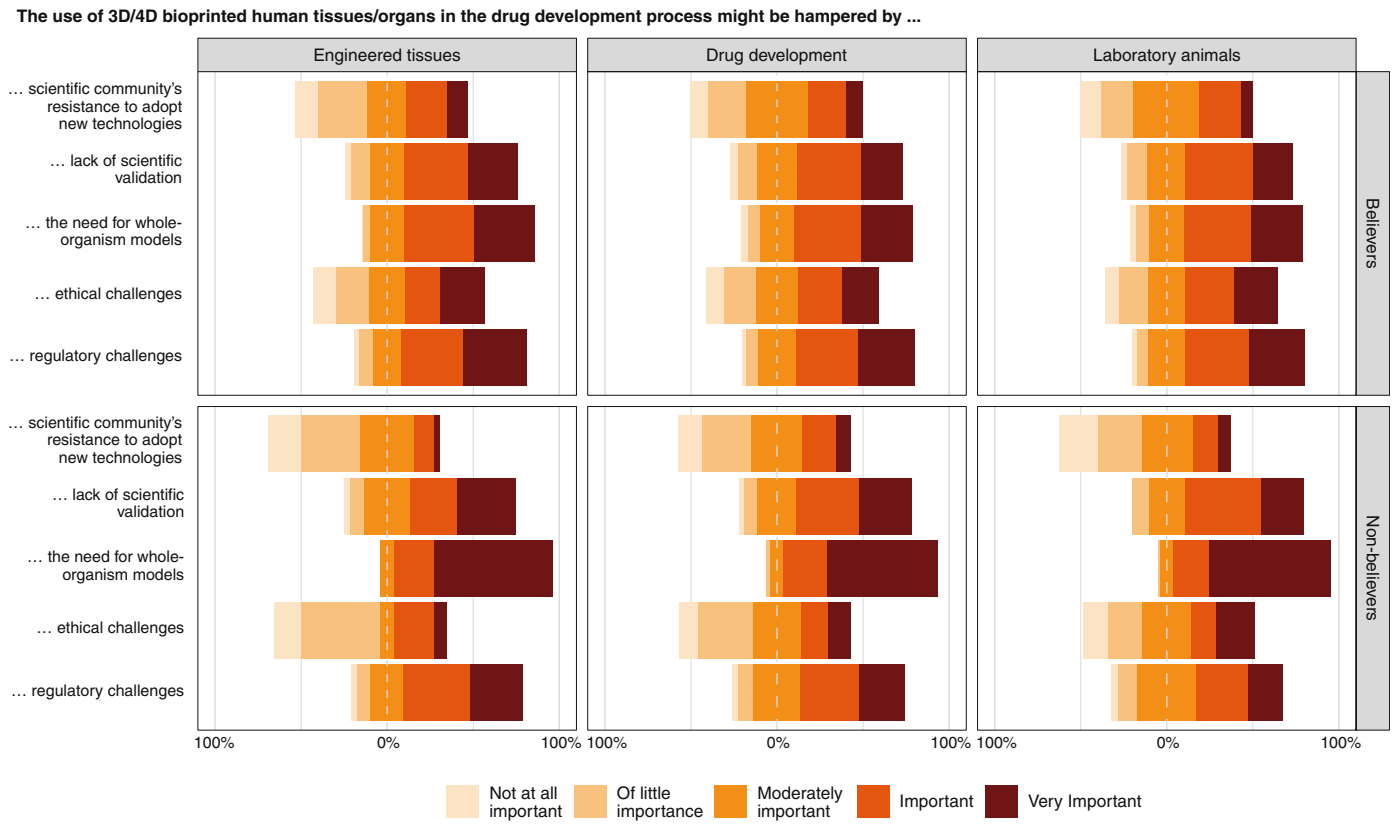

Fig. 6 | Barriers to the use of 3D/4D bioprinted human tissues/organs in the drug development process.

individuals[27,37]. If scientists can test drugs on an ever-increasing variety of tissues with more genetic variability, adverse reactions to drugs may decrease[36]. About 80% of the believers indicated that 3D/4D bioprinted human tissues/organs would likely reduce such adverse reactions. Bioprinters allowing the printing of tissues with cells from different individuals would also enable the personalized development of new drugs[27,37]. Among all respondents, this capacity for personalized drug development was regarded as one of the most well-accepted future capabilities of 3D/4D bioprinted human tissues/organs.

One of the most important pro-animal testing arguments is the need to test a drug in a whole living body[37,38]. From this perspective, the testing of new drugs on living animals helps predict toxicity in humans, thereby decreasing the risk of adverse reactions in clinical trials (and increasing success rates). Perhaps because of considerations such as these, most of the survey's respondents reported that the need to test drugs in whole organism models could hamper the use of 3D/4D bioprinted human tissues/organs in drug development.

Besides 3D/4D bioprinting, there are other new technologies currently being developed that promise to reduce or even eliminate the need for testing new drugs in whole living models. One example is the human-on-a-chip, a microfluidic platform used for testing interdependent effects of compounds in integrated miniaturized organs[37,39]. It is hoped that a combination of microfluidics and bioprinting could automate drug discovery, scaling and speeding up the process[39,40]. In our patent analysis, we found that about a quarter of the patents classified as 3D devices referred to microfluidic chips, some of which already include bioprinted tissues.

A lack of scientific validation and regulatory challenges were also pointed out as significant barriers to the use of 3D/4D bioprinted human tissues/organs in drug development. Regulatory approval of new technologies depends on scientific validation, which takes time to be obtained[41]. Tests with bioprinted models should increase over time, and if they prove to be good predictors of toxicity in humans, their acceptance by the scientific community and changes in regulations should occur naturally[39]. Overall, the expectations are that sufficient data from experiments will be available in the coming years, allowing scientists to use these bioprinted models with a high level of confidence[26]. In the early stages of their adoption by laboratories, however, these bioprinted models can only be complementary to animal testing[39].

Conclusions

Considering the reviewed literature, patent analysis, and respondents' views, it seems feasible that the use of 3D/4D bioprinted models in the drug development process will become a reality in coming years. Technology-driven changes are known to have a strong impact on the way research and development is performed. Thus, if these expectations are fulfilled, these models may not only change the way drug development is carried out, but the way research is done and taught. Facilities for breeding, rearing, and managing laboratory animals will not be needed, research protocols will be changed or abandoned, and ethical approval for drug testing may no longer be required. Consequently, universities and entities engaged in research will also need to change in order to adapt to this emerging paradigm. Preparing for the future is therefore a necessity for both the scientists involved in the advancement of knowledge and the organizations and governments supporting and financing their

activities. By forecasting future outcomes of 3D/4D bioprinted models, our study may help them prepare for the future of drug development, which, as our results suggest, is expected to be very different from the current model.

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Author contributions

F.M. designed, planned, coordinated and analyzed the work and revised the paper. L.B. reviewed the literature, interpreted results and wrote the paper. L.R. analyzed and evaluated statistical data and graphs and described results. B.C. organized and conducted the survey protocol and revised the final paper. All authors approved the submitted final version.

Competing interests

The authors declare no competing interests.

Additional information

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