Single-use Technology for the Production of Cellular Agricultural Products: Where are We Today?

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A group of the DECHEMA working group "Single-Use Technology in Biopharmaceutical Manufacture" is investigating the potential use of single-use systems for cellular agricultural applications. The preliminary results are summarized in this article.

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1 Introduction

Single-use technology currently dominates the biopharmaceutical industry [1], with a focus on vaccine and monoclonal antibody production. The single-use systems, which are currently available on the market, are used for both upstream and downstream processing, as well as for formulation and filling. Their use contributes to both a shorter development time and the intensification of commercial fed-batch production processes [2]. In addition, single-use systems can be used in the emerging field of cell therapy production, as they are able to make the mass propagation, differentiation and subsequent processing of therapeutic cells safer [3]. What is less clear is whether the single-use systems, which are already in use for the development and commercial manufacture of products, can be implemented outside the field of biopharmaceuticals, in the dynamically growing field of cellular agriculture. In addition, few studies have investigated where these systems might be used in this field in the future, whether such applications would require modifications and new development, or whether single-use systems have the potential to foster innovation in cellular agriculture.

In 2015, Isha Datar, executive director of New Harvest and co-founder of Perfect Day and Clara Foods, coined the term cellular agriculture [4]. She described cellular agriculture as the production of agricultural products at the molecular level, which correspond with traditionally manufactured agricultural products. Unlike traditional agriculture, cellular agriculture is decoupled from plants and animals. Instead, microorganisms, mammalian cell, plant cell, and plant tissue cultures are used as production organisms. The production process takes place under controlled conditions, making the final product safer and more consistent. Cell propagation and product expression in bioreactors are followed by cell separation and downstream processing steps (e.g., cell disruption, washing steps, drying processes) before product formulation and filling. Unlike traditional methods, the production process for cellular agriculture strategies not only aims to reduce its dependency on variables such as location, weather, climate and the season, but also strives to

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be more sustainable (less water and raw materials might be consumed, and exhaust gas pollution might be reduced) [5, 6]. In addition, the cellular metabolism can be influenced to develop tailor-made products which support the formation of health-promoting substances while suppressing others which are unhealthy. This process also eliminates the need for fertilizers, pesticides to control plant diseases, and antibiotics to treat animal diseases.

With a continuously growing world population and an ongoing decline in the amount of available arable land as a result of climate change, many see cellular agriculture as a potential solution to the projected supply issues that this will cause [7]. As Fig. 1 shows, cellular agriculture not only results in products for the food sector, but also ingredients for the cosmetics industry and materials for the clothing, furniture, and automobile industries. Rischer et al. [8] assign these products to 2 main product groups: (1) acellular products and (2) cellular products. Acellular products consist of organic molecules, do not contain any living cells, and are usually produced with genetically modified (GM) microorganisms. Conversely, cellular products consist of living or formerly living cells, and are produced with mammalian cell, plant cell and plant tissue cultures, usually without genetic manipulation. Both of these types of agricultural cellular products are already commercially available.

2 What Products of Cellular Agriculture are on the Market and What is Being Worked on?

Tab. 1 shows a selection of commercial cellular agricultural products, the production organisms, and their developers and applications. These are proteins, secondary metabolites, cell culture extracts, and the cells themselves, which are subsequently processed. It should, however, be emphasized that this is only a small selection of the commercially available cellular agricultural products. The majority of these are produced with microorganisms and plant cells grown in suspension [9]. Since the use of genetically modified organisms in food and cosmetic products is not generally accepted in Europe, only native production organisms are used. For this reason, the Swiss company Evolva manufactures its products in the USA, and they have only been approved for sale in the USA and Mexico so far. Likewise, a growth hormone-based cosmetics product from the company Natural BioMaterials (NBM), which utilizes a recombinant plant cell suspension-based INNOkine line, is manufactured in Korea and not in European countries.

It is striking that despite the numerous research activities concerning the production of in vitro meat, only one commercial product is currently available, namely that of the Californian company Eat Just. However, 2 new products (chicken nuggets and chicken breasts from Upside Foods and a lab-grown beef steak from Aleph Farm) have been announced for the end of 2022 [10]. In 2023, the first laboratory-produced seafood (sushi-grade piece of bluefin tuna from Blue Nalu) is expected [11].

At present, cellular agricultural products are primarily developed in the USA, Europe, and Asia. The vast majority of research activities are concerned with issues relating to the production of in vitro meat. This is the area where the most investments are currently made and the greatest challenges (cheap culture medium, new scaffolds and bioreactors for scaffolding) exist [32]. However, along with microbial milk and egg proteins, a number of other very interesting developments are currently underway for the production of food products with plant cell and tissue cultures. Examples include coffee (VTT Espoo) and also cocoa powder (Fig. 2a) (Zurich University of Applied Sciences, ZHAW, and California Cultured). The latter has been processed into chocolate bars as shown in Fig. 2b [33–36]. Also of interest are aroma compounds, which can be provided by citrus cell suspensions, for example [9].

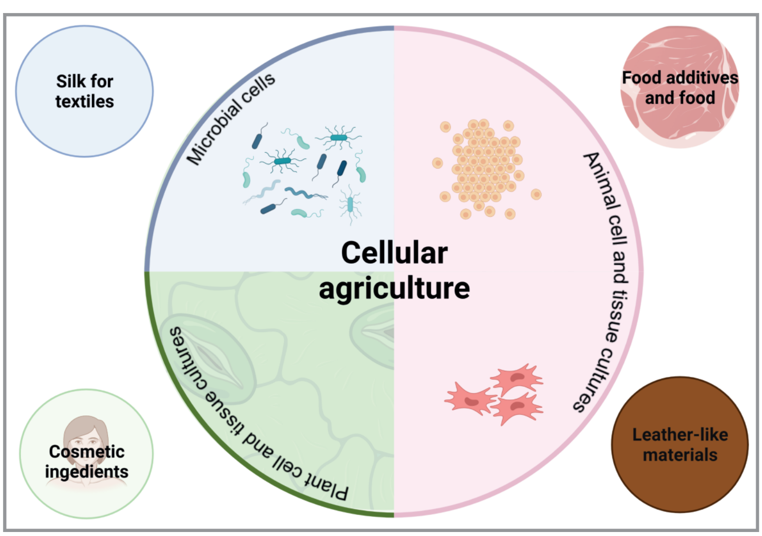

Figure 1. Selected cellular agriculture products and their production organisms. Created with Biorender.

3 Have Single-use Systems already Been Used for Cellular Agricultural Products and Why?

The development and manufacture of the above-mentioned products is already partly carried out using bioreactors with single-use technology. Interestingly, the first disposable plastic bioreactors were used for the cultivation of such cultures. In 1995, the Israeli company Osmotek launched the Life Reactor, a bubble column bioreactor [37], in which a sparger was integrated into a conically shaped plastic bag (1.5 to 5 L working volume). This bioreactor, in a modified version with a maximum working volume of 400 L, is used today by the companies Protalix

Table 1. Selected commercial cellular agricultural products.

Product	Production organism	Developer	Application	Reference
Quorn	Fungi (*Fusarium veneatum*)	Marlow Foods (now Monde Nissin Corporation, www.mondenissin.com)	Single cell protein used as meat substitute for human consumption	[12]
V-Whey	GM fungi *(Trichoderma reesei)*	Perfect Day Foods (www.perfectday.com)	Whey protein for ice creams and cow-free milk	[13–15]
ClearEgg	GM yeast[a)]	EVERY Company, formerly Clara Foods (www.theeverycompany.com)	Egg white protein for beverages, juices, energy drinks, snacks, and nutrition bars	[16]
EveNootkatone		Evolva	Flavors and fragrances for food, beverages, and cosmetics (personal and home care products)	[17]
EveValencene				[18]
SynbioVanillin		(www.evolva.com)		[19]
EverSweet			Sweetener for food and beverages	[20]
Brewed Protein		Spiber (www.spiber.inc)	Silk protein for textiles	[21]
Mikrosilk		Bolt Threads (www.boltthreads.com)	Silk protein for ties, hats, tennis clothes	[22, 23]
Zoa		Modern Meadow (www.modernmeadow.com)	Collagen for clothing, bags, shoes, cars, furniture	[24, 25]
GOOD Meat	Chicken cells[a)]	Eat Just (www.goodmeat.co[b)])	*In vitro* meat approved for human consumption[c)]	[26]
PhytoCellTec™ Malus Domestica	Suspension cells of *Malus domestica*	Mibelle Biochemistry (www.mibellebiochemistry.com)	Plant cell culture extract for cosmetics	[27]
ECHINAN 4P	Suspension cells of *Echinacea angustifolia*	Active Botanicals Research (www.abres.it)	Plant cell culture extract for food ingredients	[28]
Korea mountain ginsenosides	Adventitious root cultures of *Panax ginseng*	CBN Biotech (www.cbnbiotech.com)	Secondary metabolites for food ingredients and cosmetics	[29, 30]
INNOkine products	GM suspension cells of *Oryza sativa*	Natural Bio-Materials (NBM) (www.nbms.co.kr)	Recombinant proteins for cosmetics	[31]

a) No specification available. b) A subsidiary of Eat Just. c) Offered in Singapore restaurants and delivered to homes by Foodpanda chain.

[38] (www.protalix.com) and NBM [31] (see also Tab. 1) for the manufacture of commercial products based on plant suspension cells. These companies, however, use the culture bag several times, which is why the bioreactor is considered as a disposable, multi-use and not single-use type [39, 40].

In fact, the first upscaleable single-use bioreactor was the WAVE BIOREACTOR 20, which was developed by Vijay Singh and Marcel Röll, and was a wave-mixed bag bioreactor with one-dimensional (1D) mixing motion [41]. Since early 2000, wave-mixed bioreactors with 1D mixing motion (max. 20 L bags with 10 L culture volume) have been successfully used for the cultivation of plant suspension cultures and root cultures in the context of process developments [42, 43]. However, in order to be efficient and competitive in the production of food additives by use of cellular agriculture in the long term, bioreactors with a minimum range of at least 10 m^3 are needed. This has been determined through in-house calculations performed by experts in food technology and biotechnology at the ZHAW (personal communication T. Hühn, ZHAW, May 2022).

For the production of plant cell culture extracts for the cosmetics industry, the situation is different. In this case, a bioreactor with up to a maximum working volume of 100 L is often sufficient, which is provided by wave-mixed

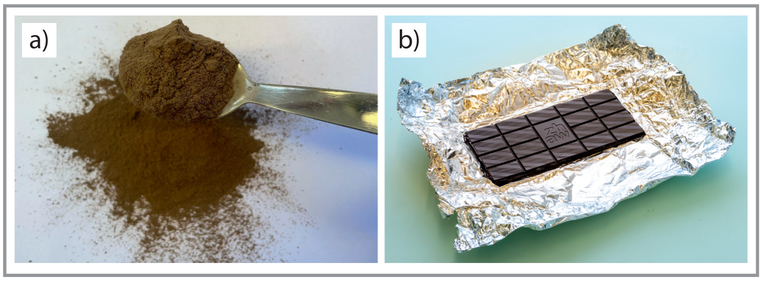

Figure 2. Future food products made with suspension cells of Theobroma cacao: a) Cocoa powder produced after cell propagation in a bioreactor and biomass separation, freeze-drying and a roasting process, b) One cell culture chocolate bar made from the cocoa powder by ZHAW specialists.

bioreactors with 1D mixing motion [44, 45]. The best-known example in this context is the production of PhytoCellTec™ Malus Domestica cell culture extract by Mibelle Biochemistry (see also Tab. 1) in Buchs, Switzerland [27]. A callus culture was initially induced from the core of an apple of the Uttwiler Spätlauber variety, from which a suspension culture was established. Following inoculum production in shake flasks, the mass propagation of the cells in 50 L single-use wave-mixed bags takes place. To produce sufficient apple cell biomass, Mibelle Biochemistry has implemented several rocker platforms with 1D mixing motion in a rocker tower (see Fig. 3a). After harvesting the biomass, the production of the liquid extract is carried out, which is accomplished using high pressure homogenization. For this purpose, reusable equipment is used. Mibelle Biochemistry manufactures its own products, such as the Zoé product line shown in Fig. 3b, and sells the extract to well-known cosmetic brands such as Lancôme and Clark's Botanicals. Through Clark's Botanicals' anti-aging serum and endorsements from Michelle Obama, this cell culture extract has become known worldwide [46]. Since its launch, companies in Europe, Korea and the USA have been developing further plant cell-based extracts for the cosmetics industry. More than 50 of such plant cell culture extracts have been launched in Europe alone in the last 15 years [9].

Special culture bags with screw caps are available to facilitate easy inoculation of and sampling with plant cell and tissue cultures in 1D wave-mixed bioreactors [42]. Their power input is generated by the rocking motion of the bioreactor platform, which generates a wave in the fixed bag containing the cells and the medium. In this way, mixing occurs with homogeneous energy dissipation and a more homogeneous shear stress pattern than in stirred cell culture bioreactors [47]. Furthermore, the surface of the medium is continuously renewed, and bubble-free surface aeration occurs. This ultimately results in less foaming, often makes the addition of an antifoam agent unnecessary, and simplifies downstream processing. Nonetheless, in the case of very fast-growing plant suspension cells, whose growth is accompanied by a significant increase in viscosity, the specific power input and the oxygen transfer may result in limitations in wave-mixed bioreactors with 1D mixing motion [38, 48]. This has been described by Eibl et al. for a plant cell culture broth with non-Newtonian flow behavior [49, 50]. For such cell culture broths, which also include resveratrol-producing grape suspension cells, wave-mixed bioreactors with 2D mixing motion such as the CELL-tainer may be more suitable [9, 38, 51].

Various publications include bioengineering parameters such as mixing time, k_La, fluid flow, specific power input, and shear stress for the CELL-tainer [52–56]. However, at this stage, the data on the bioengineering parameters of this type of wave-mixed bioreactor cannot be compared with those for wave-mixed bioreactors with 1D mixing motion. The reason is the different rocking motion, which leads to an additional acceleration of the fluid in the horizontal direction while flowing from one side of the bag to the other. The higher k_La value allows for the application of aerobic microbial cultures. In the case of filamentous organisms, the evolvement of a certain macromorphology can be achieved through media supplements, as shown for *Aspergillus niger* [57]. Shaken single-use bioreactors exhibit several advantages over stirred-tank reactors if certain macromorphological structures are being obtained, such as in filamentous organisms. The mechanical shear-forces are an order of magnitude lower than in stirred systems. This affects growth and the macromorphological development, it changes the size and constitution of filaments and even makes their evolvement controllable. It allows for the growth of filaments on a textile structure under shaking conditions, leading to a kind of fabric that can replace wool [58].

It is worth mentioning, that a 2D mixing motion has also been successfully used by Mosa Meat for the mass propagation of bovine satellite cells on microcarriers [59]. These muscle cells are differentiated into muscle fiber cells in a subsequent step. Together with adipocytes, these are used for the production of minced in vitro meat.

In general, if rapid process development (screening studies, scale-up) is required, the user

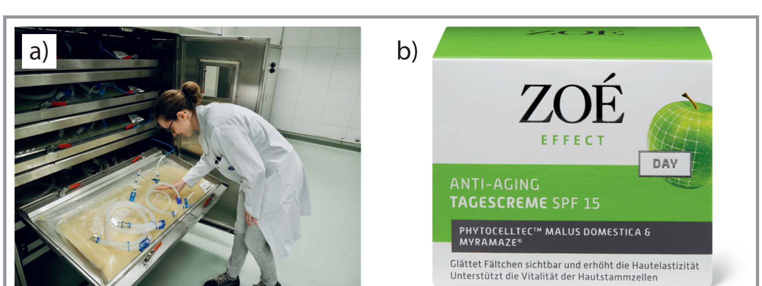

Figure 3. PhytoCellTec™ Malus Domestica. a) Example of the rocker tower at Mibelle Biochemistry, in which apple suspension cells are mass propagated in single-use wave-mixed bags, b) Zoé product line sold by Migros in Switzerland (Courtesy of Mibelle Biochemistry).

has other options in addition to wave-mixed systems. For animal cell cultures and microorganisms, but also in some cases for plant cell cultures, instrumented, orbitally shaken and stirred single-use bioreactors are suitable from the mL-range to pilot scale (Tab. 2) [51, 60].

4 How Can Single-use Technology be Applied More Extensively to the Development and Production of Cellular Agriculture Products?

The wide range of commercialized single-use systems is exclusively comprised of equipment that is designed for the manufacture of biopharmaceutical products. In the case of commercial processes, these must be carried out in compliance with good manufacturing practice (GMP). This means that the manufacturing processes, including those which utilize single-use equipment, are subject to their corresponding guidelines and regulatory documents. Once the equipment has been qualified depending on its use, the production process also needs to be validated. A substantial amount of effort is required to produce and qualify GMP-compliant single-use systems and in particular the disposables that need to be renewed. This is ultimately reflected in the high price of the disposables.

The aforementioned focus of disposables in single-use systems and their associated high price currently hinder their use beyond the biopharmaceutical sector. The advantages of using single-use technology, however, have been published in numerous studies (higher bio- and process safety, faster process and product changes, lower basic investment, lower infrastructure and maintenance costs, lower footprint, sustainability despite plastic waste and design variety). These demonstrate the feasibility of its use in cases where biotechnological production processes need to be implemented quickly, safely and sustainably.

This would include many developments and commercial production processes for cellular agricultural products, but a food-grade version of the disposables would be perfectly adequate. Such a disposable could lead to the use of cheaper plastic and film materials as well as cheaper production. Furthermore, in comparison to pharma-grade disposables and assuming appropriate quantities, lower prices can be expected for food-grade disposables. The user would then have the opportunity to transfer the existing single-use equipment and the know-how acquired manufacturing in various areas of application over the years to cellular agriculture. This mainly concerns processes with mammalian cell, plant cell and plant tissue cultures and, to some extent, also those with microorganisms. Furthermore, the multiple use of disposables as already implemented by Protalix and

Table 2. Overview of instrumented, orbitally shaken and stirred single-use bioreactor systems which are relevant to process developments in conjunction with cellular agricultural products.

Bioreactor	Working volume [L]	Organism	Reference
BioLector[a)]	0.0005	Plant suspension cells	[61, 62]
		Microorganism	
BioBLU[b)]	0.15	Microorganism	[63, 64]
		Mesenchymal stem cells[c)]	
Ambr 15/250[b)]	0.015/0.250	Microorganism	[65–67]
		Mesenchymal stem cells[c)]	
UniVessel SU[b)]	2	Mesenchymal stem cells[c)]	[68–70]
		Fungi	
		Plant suspension cells	
BactoVessel SUF[b)]	2/4/12	Microorganism	[71]
BIOSTAT CultiBag STR[b)]	50	Microorganism	[68, 72]
Mobius CellReady[b)]	50	Mesenchymal stem cells[c)]	[73]
HyPerforma S.U.F[b)]	30/300	Microorganism	[74]
HyPerforma S.U.B[b)]	50	Plant suspension cells	[75]
SB50-X[a)]	50	Plant suspension cells	[75]

a) Orbitally shaken. b) Stirred. c) On microcarriers.
Remark: Satellite cells used to produce in vitro meat behave similarly to mesenchymal stem cells in terms of morphology and growth [76]. Therefore, suitable bioreactor systems for the latter have also been included in the table.

NBM with the culture bags of the bioreactors used should be examined as an alternative (see also point 3). It should be investigated whether there are process steps in the manufacturing process that allow for the multiple use of disposables without impairing product quantification, quality, or process safety.

For the production of cellular agricultural products, single-use systems in upstream processing are suitable for small and medium product volumes and during seed cultivation. Sterile, pre-qualified single-use filters, storage containers with bags, single-use mixers, single-use bioreactors and single-use separators guarantee the aseptic conditions required for upstream processing with drastically reduced cross-contamination. In fact, they allow for the design of hybrid single-use production facilities for cellular agricultural products. For large product volumes, at a minimum, the implementation of inoculum production with single-use technology should be considered. Wave-mixed bioreactors have the advantage of a wide volume range which can be applied in a single bag, which can reduce transfer steps and thus, save costs and reduce contamination risks. In addition, the advantages and disadvantages of continuous cell and product expressions using perfusion techniques should be investigated. Perfusion techniques can allow for a reduction in scale by a factor of 10 or more and are currently experiencing a renaissance in the biopharmaceutical field. It should be noted that for some cellular agricultural products, hygienic, but not aseptic conditions are required after harvesting. Open filling processes are an exception here.

However, the downstream processing of cellular agricultural products (biomass as product, culture or fermentation broth as product, intracellular product) often does not correspond to that of biopharmaceutical products. As mentioned in the first section, single-use equipment exists for the downstream processing of biopharmaceutical products (storage bags with associated bioprocess containers, mixers, filtration systems, membrane adsorbers, chromatography systems, freeze-and-thaw systems, etc.), but single-use systems for downstream processing relevant to the production of food additives or cosmetic active ingredients (kneaders, extruders, boiling and blanching apparatus, high-pressure homogenizers, etc.) do not currently exist. They are, however, mandatory for complete single-use production facilities.

5 Outlook

Single-use technology might become significant in the future, especially for operating small, decentralized production facilities for cellular agricultural products. Single-use systems are easy to operate, do not require fixed piping or a steam supply for their operation, and are already available on the market up to 6 m^3. Therefore, the long-term use of this technology is quite feasible on farms, in craft stores, small businesses, restaurants, or even at home. Their design variety allow for many potential applications. This could be, for example, single-use bioreactors in which animal cells for meat production are propagated in perfusion mode by the farmer, or plant cell biomass that is produced in a simple single-use bioreactor at home to prepare a smoothie or cereal in the kitchen. However, to accomplish this, the disposables that need to be innovated must first be made more affordable, and the potential for multiple uses has to be investigated. To date, the use of single-use systems in processes for cellular agricultural products is limited to rapid product developments (e.g., in cell line and parameter screening) and the commercial production of high-value products on a scale of up to 100 L working volume for the cosmetics sector, but the potential for a broader range of applications exists.

Abbreviations

GM	Genetically Modified
GMP	Good Manufacturing Practice
NBM	Natural Bio-Materials
ZHAW	Zurich University of Applied Sciences

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