Presentation Title:

The Bolt-on Bioreactor project - Perfusion bioreactor design for efficient adherent cell culture.

The Bolt-on Bioreactor Project

Adherent cell culture system

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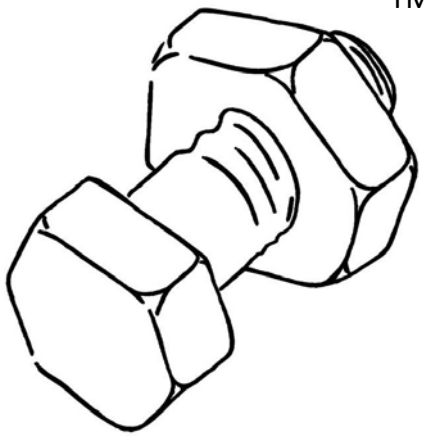


Abstract:

Efficiency of bioreactors for the culture of adherent cells lags behind that of their suspension cells counterparts.

The Bolt-on Bioreactor (BoB) project has studied each of the four major challenges that preclude adherent cells from becoming the biopharmaceuticals production system of choice in industry. The result is an efficient and scalable system for the perfusion culture of adherent cells.

This document is a transcription of the presentation made by Marcos Simón, founder of The Bolt-on Bioreactor project, at the 7th annual Protein and Antibody Engineering Summit, PEGS Europe. 2-6 November 2015, Lisbon, Portugal.

Hyperlinks to extended content included

Bacteria, yeast, fungi, insect cells, plant cells and mammalian cells are the most widely used cell types for the production of biopharmaceuticals.

The largest market share of biopharma medicines is produced in mammalian cells cultured in suspension, while adherent mammalian cells do not represent a large market share despite their superior productivity per cell and simplicity of use.

This presentation explains the understanding of The Bolt-on Bioreactor project of the reasons for the prevalence of mammalian suspension cells in biopharmaceuticals production and describe the design features of the Bolt-on Bioreactor, a bioreactor specially tailored to overcome the productivity deficiencies of current adherent cell culture systems.

Host cell types used in biopharmaceuticals production

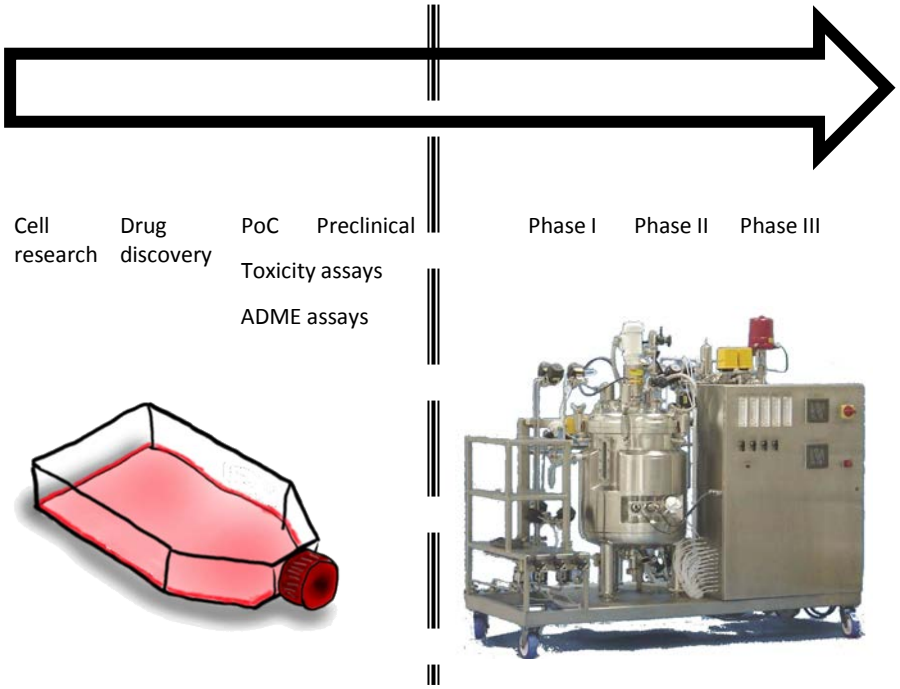
- Bacteria (*E. coli*)
- Yeast
- Fungi
- Insect cells
- Plant cells
- Mammalian cells
  - Suspension cells
  - Adherent cells

When we look into the uses of adherent and suspension cell culture, we see that in general, adherent cells are the basic research and discovery tool.

A common route for biopharmaceutical development is that basic research, drug discovery, proof of concept and preclinical studies are carried out using adherent cell culture, but when production scale increases, at the start of Phase I studies, the production system is normally adapted into suspension cell culture.

Now we will analyse the reasons that drive this trend.

Transition from adherent to suspension cell culture

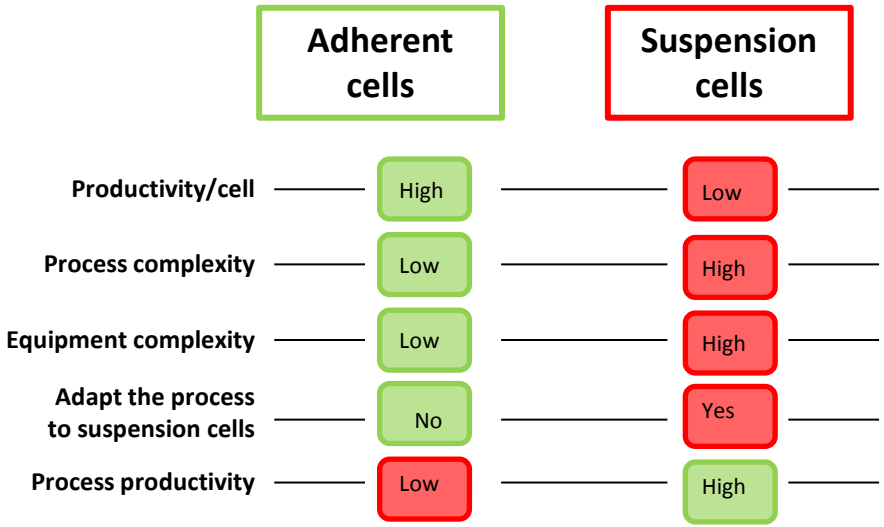


These comparison parameters are relevant to the biopharma industry: Productivity per cell, Process complexity, Equipment complexity, Adaptation of the process to a different cell type and Process productivity.

We see that Productivity per cell, Process complexity, Equipment complexity and the need to Adapt the system to a different cell type, are all of them parameters in which adherent cells outperform suspension cells.

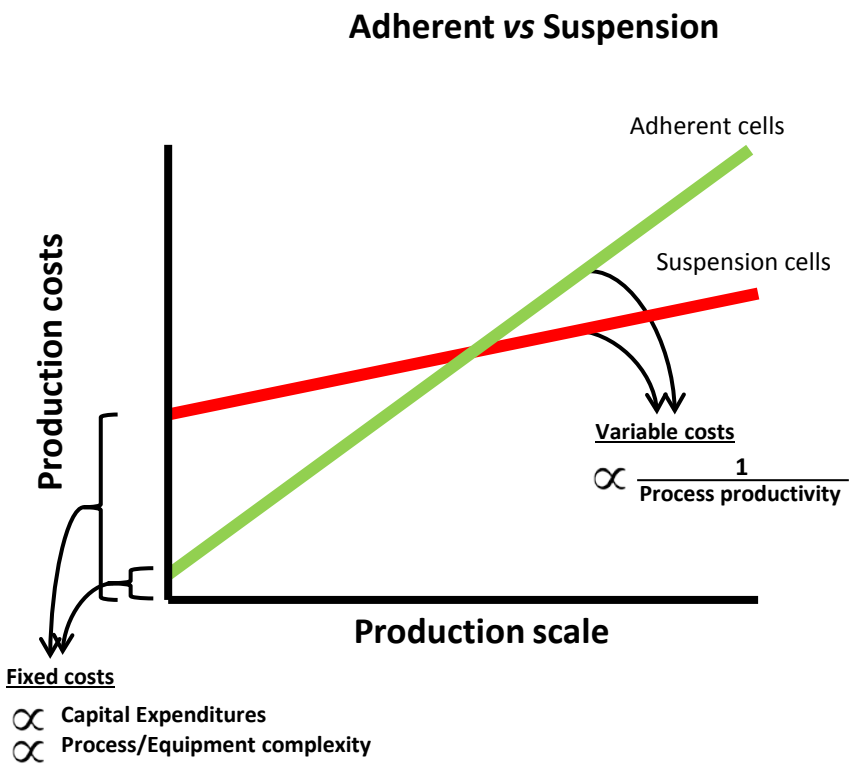
However, overall process productivity is more favorable to suspension cells. And this is the key.

Adherent *vs* Suspension



As a result of this comparatively higher process productivity, variable costs are lower for suspension cells.

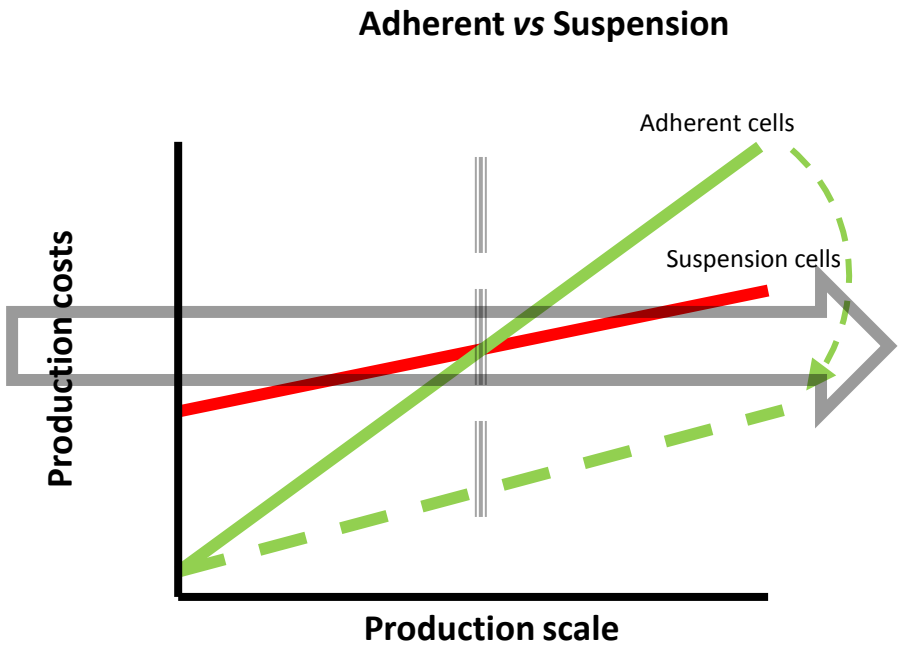
And despite the fact that fixed costs are in general significantly higher for suspension cells due to Capital Expenditures and process and equipment complexity, the overall production costs make suspension cells more favorable, economically, at scales above a certain production volume.



As it happens, this transition point is more or less coincident with the start of Phase I studies, and explains why adherent cells are abandoned in favor of suspension cells systems at larger scales.

Certainly there are some exceptions since adherent cell culture is also used in some industrial production processes. These exceptions include production for cell therapy, highly active biopharmaceuticals, some vaccines and those processes where strain constrains preclude the change.

The purpose of The Bolt-on Bioreactor project is to develop a cost-effective system for the culture of adherent cells, and therefore, we had to reduce variable production costs by increasing process productivity, which in our view means increasing volumetric productivity and automating production systems.



Exceptions: cell therapy products, low scale production, some vaccines strain constrains

We have studied available culture systems for adherent cells and analyzed the weaknesses of each system.

We have concluded that a successful alternative to existing devices must solve four major challenges. The first challenge has to do with volumetric productivity, the second with process automation, the third with containment and sterility, and the fourth with process economics.

In the following pages we comment on each of these challenges and then explain what our proposal to solve them is.

The four challenges

Challenge #1 – Volumetric productivity

Challenge #2 – Process automation

Challenge #3 – Containment and sterility

Challenge #4 – Process economics

In adherent cell culture, volumetric productivity is directly dependant on the amount of cell attachment area available per unit of volume.

We arbitrarily decided to set the required volumetric productivity to a 10 times increase with respect to available systems.

Besides, the surface provided should be suitable for cell anchorage and growth.

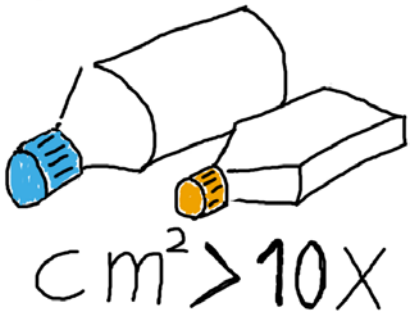
And the resulting cell culture device should be of a size that a laboratory technician can handle with ease.

Challenge #1 - Volumetric productivity

> 10 X cell culture area

Suitable surface for cell attachment

Handable cell culture device



The second challenge, related to Process automation, is to develop a system that can automate a continuous culture process for anchorage-dependent cells while preventing cellular stress cycles through a stable culture environment that avoids discontinuous medium replacement and intermittent cell detachment.

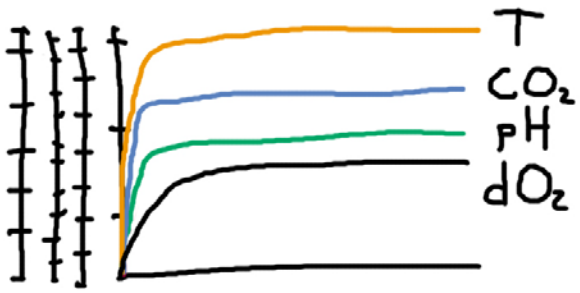
The system should provide homeostatic culture conditions throughout a culture process with programming and manual control options to modify culture conditions.

Challenge #2 - Process automation

Automated and continuous process

Stable culture environment

Programable and traceable



The third challenge is to develop a system in which adherent cells are cultured in a contained chamber, and where contamination risks are minimized even when the system is operated in non-classified laboratories.

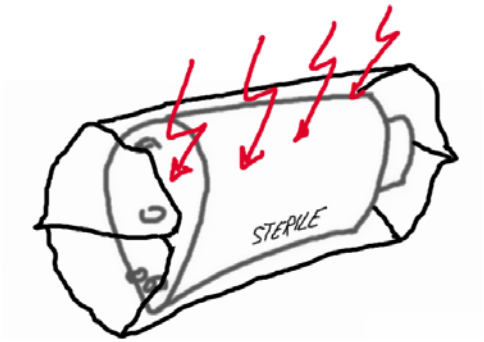
This system should be provided sterile and ready to use, and when in use, it should protect cell cultures from contamination by the surrounding environment.

Challenge #3 - Containment and sterility

Contained culture process

Reduced contamination risk during handling

Sterile, ready-to-use cell culture device



And the fourth challenge has to do with process economics.

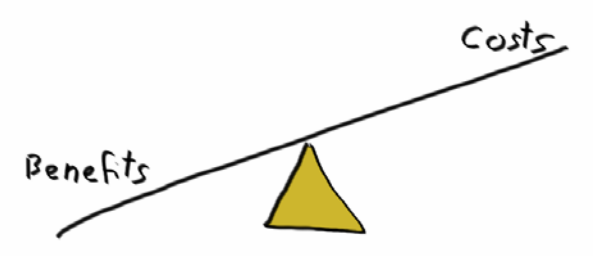
This challenge means that the system should be economically feasible to provide economic benefits both to the vendor and to the customers.

Challenge #4 - Process economics

Economic feasibility

Profitable for vendor

Profitable for customer



To find a solution to the volumetric productivity challenge, we analyzed different strategies to increase cell attachment area while bearing in mind the need to provide a suitable attachment surface for monolayer growth and an easy to handle culture device.

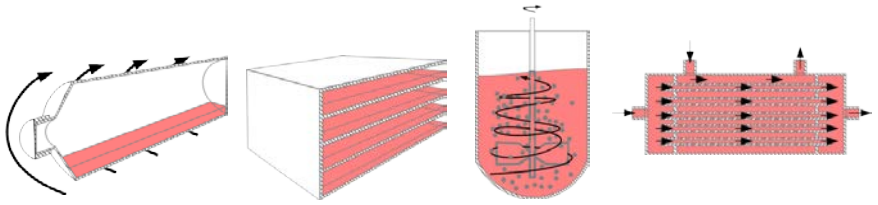
We studied the weaknesses of available systems and after comparing roller bottles, plate stacks, microcarriers and hollow fibers, we decided that the best solution to challenge #1 was a rolled membrane within a closed reservoir where cells can attach to both sides of the whole membrane, thus achieving huge cell density within the same volume of current culture devices.

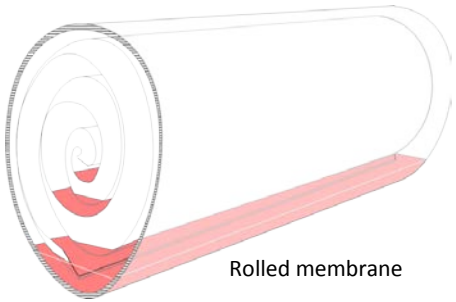
By controlling the number of turns in the membrane, we can control the amount of attachment area available to the cells. But to make this system useful it is necessary to provide a uniform liquid distribution system that ensures complete access of the cells to the entire membrane and to automate culture medium and gas replacement to ensure proper availability of nutrients to the cells.

The picture to the right shows one of our prototypes. You can see the liquid flowing from the inner layer of the rolled membrane. You can watch a short video on our web page.

Video available at: http://www.boltonbioreactor.com/the-bob/the-four-challenges/generica.html
Also available at: https://www.youtube.com/watch?v=YP0RDTVS80s

The BoB solution to Challenge #1 (Volumetric productivity)



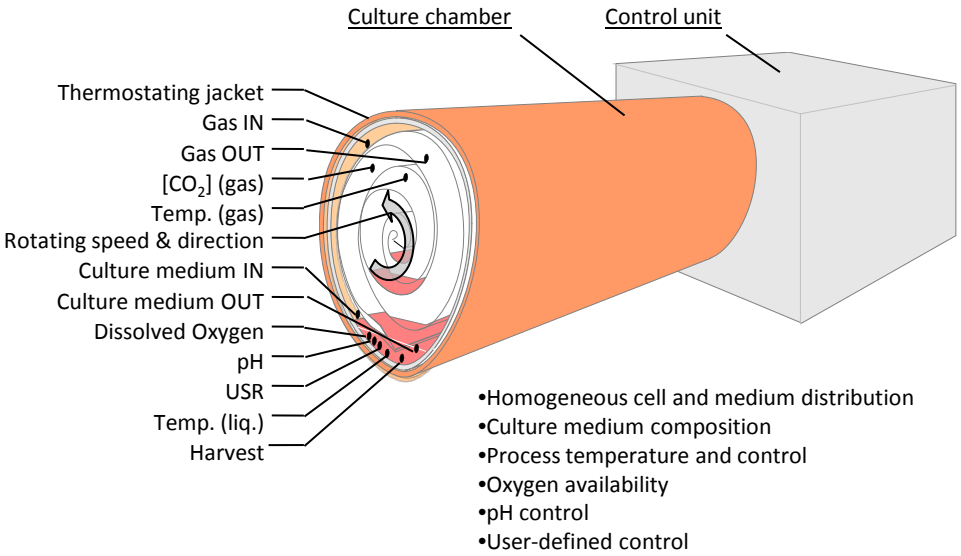


BioProcess International 13(1) January 2015
Available at: http://www.boltonbioreactor.com/about/media-coverage.html
Also available at: http://www.bioprocessintl.com/

Following our analysis of the adherent cell culture process and available commercial systems, we concluded that a reliable automated and continuous culture process for adherent cells based on a rolled membrane system requires control of:

- Homogeneous cell and medium distribution, achieved through controlled rotation speed and direction of the rolled membrane.
- Control of culture medium composition is achieved through medium replacement rate via the Culture medium IN and OUT ports.
- Process temperature is controlled by the combined effect of a thermostating jacket, a culture medium reservoir in the bottom of the device, and the heated gas inflow.
- Oxygen availability is ensured by the continuous gas inflow and the large volumetric mass transfer coefficient of the system.
- pH is controlled by CO_2 content in the process gas and by culture medium replacement. Pumps will be available for introducing acids and alkalis if required.
- Additional user-defined controls will also be available based on customized probes.

The BoB solution to Challenge #2 (Process automation)



BioProcess International 13(4) April 2015
Available at: http://www.boltonbioreactor.com/about/media-coverage.html
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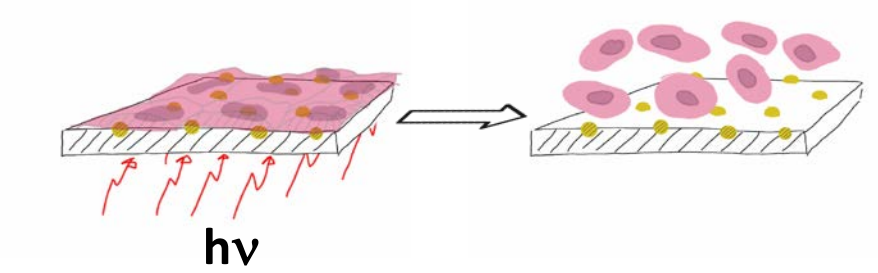
In addition to the previous features, we are currently developing a unique, non-invasive, cell harvesting technology based on plasmonic cell culture substrates.

This astounding harvesting method is based on irradiating the device with a near-infrared light source. When the light reaches this specially tailored support, it interacts via plasmonic phenomena with special nanoparticles embedded in the culture surface. As a consequence of this interaction the particles are locally heated and promote detachment of the cells from the attachment surface.

The BoB solution to Challenge #2 (Process automation)



Non-invasive cell harvesting based on plasmonic substrates

Patent pending

CIC biomaGUNE
www.cicbiomagune.com

Juan José Giner *et al.* Plasmonic Surfaces for Cell Growth and Near-Infrared Light Triggered Retrieval. 2015. *Angew. Chem. Int. Ed.* In press

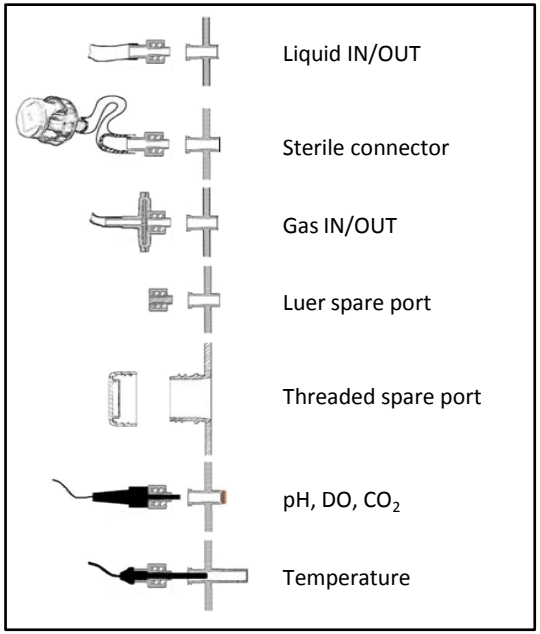
In relation to the containment and sterility challenge, we decided that the culture device should be provided pre-sterilized and ready-to-use.

To achieve this goal, we decided that gamma or beta irradiation was the most appropriate sterilization method and to do this, we endeavored to design a device entirely made out of plastic.

For the connection of fluid lines and probes we decided to use luer connectors as the default option because of the large number of compatible connectors and accessories in the market. The BoB culture chamber will also have a spare threaded port for special uses.

We will use non-invasive probes to avoid potential contamination coming from connecting probes.

The BoB solution to Challenge #3 (Containment and sterility)



BioProcess International 13(5) May 2015
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A particularity of this system is how we have solved the issue of agitating the rolled membrane within the culture device. Due to the chosen sterilization method, we don't want to use metallic elements within the culture chamber, we want tight control of the rotational movement, and we want a static culture chamber to which probes and tubes can be connected for continuous fluid transfer while the system remains fully contained.

We came up with an agitation system based on mechanical coupling of plastic bearings within the culture chamber accommodated in corresponding cavities between bearings on a driving disc outside the chamber, on the control unit. The bearings inside the culture chamber are separated from those in the control unit by a flexible membrane that provides containment without interfering with the mechanical coupling.

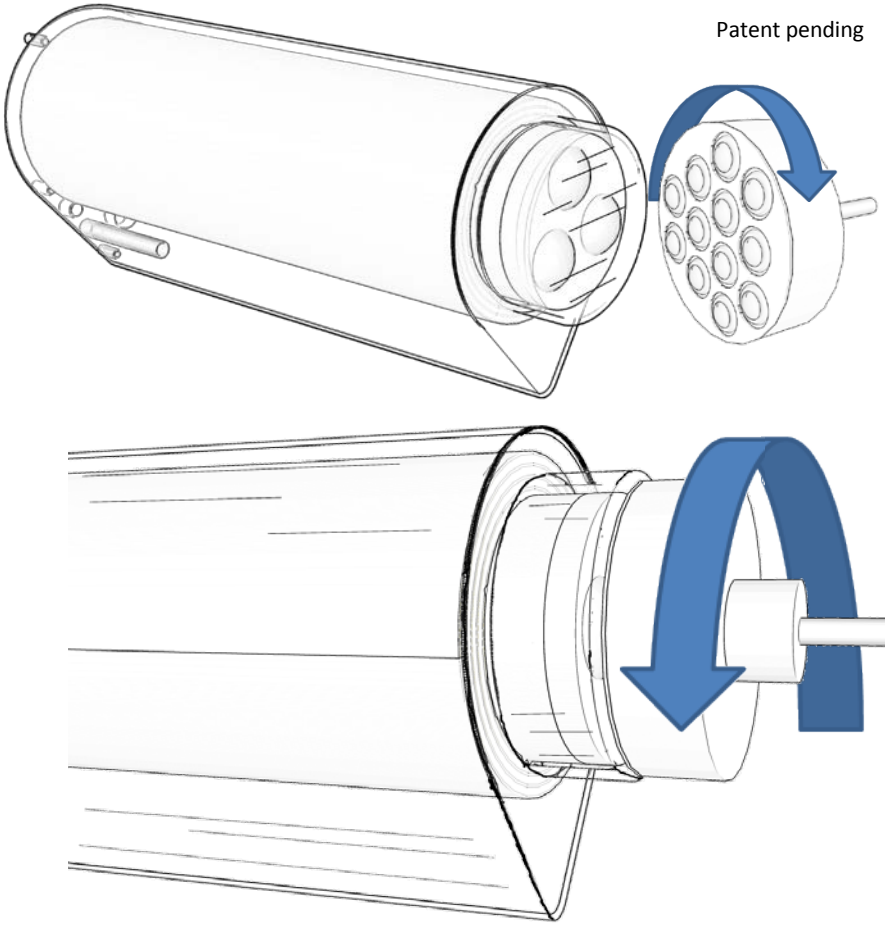
Because the bearings inside the chamber are located in a disc that is integral to the rolled membrane on which cells attach, the rotational movement of the driving disc in the control unit conveys motion to the rolled membrane, providing for a system with nonintrusive agitation

This design allows for the intercalation of an additional flexible membrane that is integral to the laboratory wall in such a way that the control unit can be placed outside de cell culture laboratory while the culture chamber is inside the laboratory, thus minimizing extrinsic contamination risk during use.

Video available at: http://www.boltonbioreactor.com/the-bob/the-four-challenges/generica_3.html
Also available at: https://www.youtube.com/watch?v=o_VMwmcICxc#t=33

The BoB solution to Challenge #3 (Containment and sterility)

Patent pending



In order to find a solution to challenge four, related to process economics, we had to estimate what public prices for both, the culture chamber and the control unit make the BoB system profitable both to vendor and to customer compared to competing technologies.

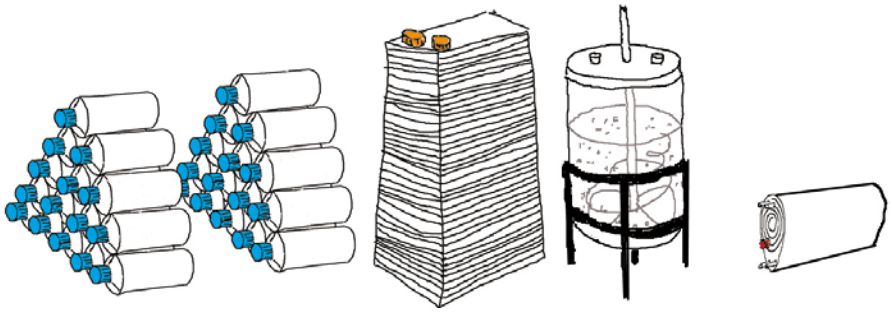
To do this we compared production costs and capital expenditures necessary to carry out adherent cell culture at different process scales using each of these technologies. Process scale in this analysis is measured as the amount of cell attachment area available

This example illustrates the different amounts of culture devices necessary to culture adherent cells in 25.000 cm^2 depending on the chosen technology. You see, for example, that 30 roller bottles are necessary to provide a culture area equivalent to a single BoB culture chamber.

Production costs such as culture medium usage, price of cell culture device, labor and accessories, and investments, are different for the different technologies and at different process scales some technologies present advantages over others.

The BoB solution to Challenge #4 (process economics)

25,000 cm^2 of attachmet area



30 X roller bottle
(850 cm^2/bottle)

1 X 4 l microcarrier bioreactor
(20 g/l; 320 cm^2/g))

1 X 40-layer plate stack
620 cm^2/layer)

1 X BoB
(25,000 cm^2/CC)

In our analysis we included only costs and expenses that are affected by the chosen cell culture technology. This is, if a particular investment or cost is the same no matter what technology we use, this parameter is excluded from the analysis.

And the analyzed process spans the initiation of cell culture to total colonization of all available culture surface plus a further 15-day maintenance culture for continued supernatant harvesting.

Then we computed all collected data and compared process costs per cm^2 when the price of the BoB culture chamber is zero. We also compared all capital expenditures, related to production, when the investment in BoB control units is zero.

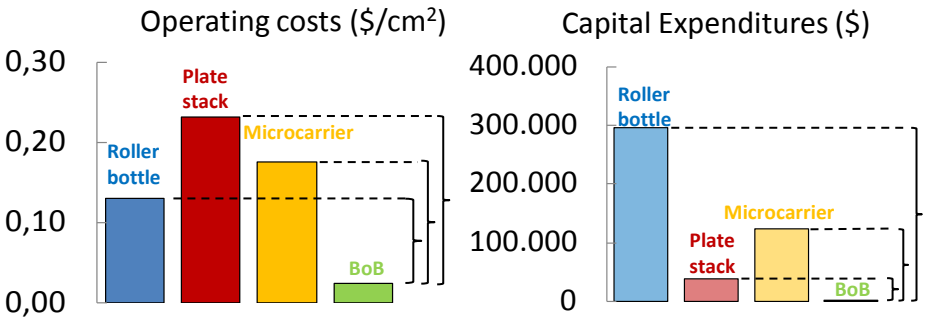
By comparing operating cots per cm^2 of a given technology against BoB when the price for the BoB culture chamber is zero, we obtain the maximum public price for the culture chamber that makes the BoB technology economically more advantageous for the customer than competing technologies.

And by comparing the capital expenditures necessary for a given technology against BoB when the price for the BoB control unit is zero, we obtain the maximum investment in BoB control units that still makes the BoB economically advantageous to the customer compared to competing technologies.

All this for a given scale. 1,000 m^2 in this example.

The BoB solution to Challenge #4 (process economics)

Comparison at 1,000 m^2 (10 x 10^6 cm^2) production scale



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We repeated this analysis at six different process scales. The numbers in the left-hand table indicate the maximum price per cm^2 for the BoB culture chamber that makes operating costs cheaper for the BoB system compared to competing technologies at a given scale.

Our findings show that, except for a couple of exceptions (marked light red), BoB culture chambers can be sold at prices per cm^2 well above the prices of competing devices, and still make the process costs economically beneficial to the customer.

Numbers in the lower table indicate average public prices per cm^2 of roller bottle, plate stack and microcarrier, respectively.

In the right-hand table we see how much the customer can invest in BoB control units and still make savings in capital expenditures related to facilities set-up compared to other technologies.

Video available at: http://www.boltonbioreactor.com/the-bob/the-four-challenges/generica_4.html
Also available at: https://www.youtube.com/watch?v=lbhjqnJ9w4U#t=25

The BoB solution to Challenge #4 (process economics)

Allowable public prices for the BoB system

BoB culture chamber ($/cm²)

Production scale (m^2)	Roller Bottle	Plate Stack	Micro-carrier
0.1	-0.955	-0.558	0.783
1.0	-0.025	0.165	0.246
10	0.054	0.151	0.149
100	0.087	0.169	0.147
1,000	0.106	0.209	0.152
10,000	0.103	0.214	0.145

BoB control units ($)

Production scale (m^2)	Roller Bottle	Plate Stack	Micro-carrier
0.1	6,652	5,632	8,351
1.0	9,075	7,355	15,919
10	18,267	9,989	30,424
100	56,116	15,732	58,828
1,000	293,176	35,414	120,783
10,000	2,260,807	174,755	320,789

Average price ($/cm²) of competing devices		
Roller bottle	Plate Stack	Micro-carrier
0.017	0.061	0.044

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Having found sound solutions to all four challenges, we decided to start product development of the BoB.

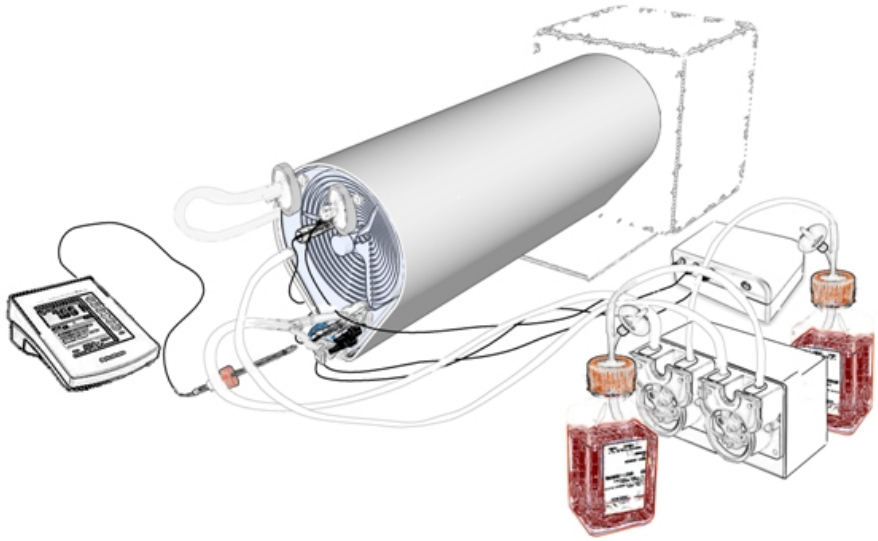
This image shows what the BoB will look like once we finish product development. You can see the culture chamber coated with the thermostating jacket, the control unit, gas filters, probes, peristaltic pumps, signal readers, culture medium and waste.

Currently we are focused on the development of the culture chamber and the control unit. And the design of the bench-top version of the BoB shows the intensive use of commercially available accessories that we will be doing.

We are intentionally adopting the most extended standards to facilitate adoption of the technology by established laboratories.

Here you will find a more detailed description of the design:
http://www.boltonbioreactor.com/the-bob.html

The Bolt-on Bioreactor



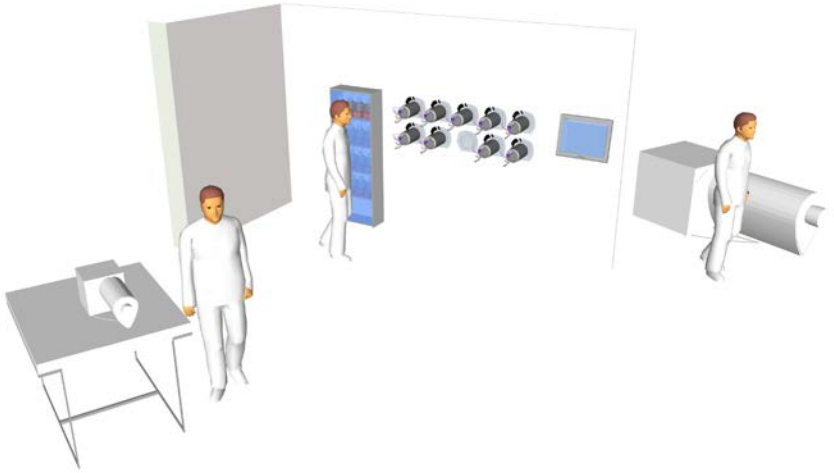
And this is our vision of the different embodiments of the commercial product.

To the left you see a laboratory scale system, meant for process development and low scale production, up to 50,000 cm^2.

In the middle a multi-unit system with the control units located outside the culture laboratory. Control unit and culture chamber in this embodiment are separated by a flexible membrane integral to the laboratory wall.
This represents a therapeutic cells production laboratory.

And to the right you see a large scale production unit meant for industrial production, with a capacity of up to three million cm^2.

The Bolt-on Bioreactor



A final remark on the progress of this endeavor is that The Bolt-on Bioreactor project has been very much delayed due to lack of funding.

Still, we have made a successful proof of concept of the technology and currently we are developing the product.

Soon, when we end production of evaluation units we will start an evaluation trial with representative end-users.

The Bolt-on Bioreactor project – current status

-Succesful Proof of Concept
-Product development ongoing
-End user evaluation trial coming soon

We are looking forward to attending your cell culture needs.

Thank you very much for reading!

The Bolt-on Bioreactor team :-)
www.boltonbioreactor.com

The Bolt-on Bioreactor Project

Adherent cell culture system

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