3D Printing of Biocompatible Materials Targeted for the Skin: Technological Survey Report and Business Intelligence

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Abstract

Bio-printing is a revolutionary technology in the world of tissue engineering, it enables the printing of cells at highly variable scales and resolutions for a wide range of applications including skin grafting and cosmetic testing. This paper proposes to make an inventory of the bio-printing applied to the creation of skin, taking the technical and economic aspects related to skin printing. The scope of this study will focus on Europe with a perspective on the world.

Introduction

Beginning in the 1980s, 3D printing quickly established itself as a promising technology. Nowadays, this process excels in creating complex and detailed three-dimensional structures from inert materials such as plastics or metals at a low cost. Moreover, innovative inks are used to functionalize the objects created so that they acquire or retain certain properties during their solidification.

One of these applications is about tissue engineering : the creation of living tissue artificially. Bio- printing goes beyond a simple deposition of layered cells and allows the creation of interactions between cells until it comes to almost functional tissue. This innovation is active and widely funded by Europe. The stake is therefore great and recognized.

Whether in the context of transplants, in order to compensate for the lack of donations, or in case of cosmetic skin tests, in order to get ahead on regulatory changes and to offer an ethical and economical alternative; the bio impression of the skin brings many advantages.

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In this document, bio-printing applied to the skin will be studied. You could find a technical state of the art of the different technologies used to print organs and they will be compared. Then the economical context surrounding bio-printing will be described. The scope of this study will focus on European innovations with an opening to the world.

We will try to answer the following questions : what technologies are suitable for skin printing ? Is bio-printing an economically and ethically viable solution for organ transplants ? Will the cosmetic testing industry lead to innovations in the printed skin sector ? How is the bio-printing market developing ?

1. Scope

1.1. From printing to bio-printing

3D printing was born in the 1980s thanks to the company CILAS-Alcatel. The principle is to generate an object from a polymer deposited in layers. In contrast to conventional machining processes, 3D printing is additive manufacturing where the material isn't sliced, which limits waste generation. [1] Many applications have emerged in different fields with the evolution of this technology, in particular the printing of living tissues: bio-printing. This application is very recent since the first printed organic creation was made in 2003 by Thomas Boland. [2]

Bio-printing is the application of 3D printing to the spatial structuring of cells and biomaterials (here living tissue) using layer-by-layer deposition according to predefined organizations by digital design. Beyond simple layers of cells, one of the promising applications of bio-printing is the creation of organs and tissues.

1.2. Bio-printing supporting organs transplant

The development of bio-printing has a real scientific interest for the general understanding of the influence of the microenvironment on the living system. It would provide a more complex understanding of the functions of living tissue. The final goal would be the synthesis and thorough mastery of organ transplantation. [3]

1.2.1. Tissues and organs transplants

An organ is a part of the body that performs one or more specific biological functions, relying on several tissues organized in a complex structure. A tissue is made up of similar cells, all having the same function (eg skin, muscle, bones, etc.).

A transplant is a surgical act replacing a failing element (organ or tissue) of the human body with a functioning one. This technique is considered by medicine when no other treatment is effective, but transplantation is now a usual remedy. [4]

The transplant is the general term for the part of the human body to be transplanted (organ, tissue or cells).

1.2.2. Rejection of transplant

The major histocompatibility complex (MHC) is the set of molecules carried on the surface of a cell and characteristic of an individual, allowing the recognition of the "self" by the immune system.

Since MHC is different from one individual to another and like blood groups for transfusion, it is necessary to ensure that MHCs are as close as possible between donor and receiver before a transplant. However, given the complexity and variability of MHC, the correspondence is never perfect (except between identical twins). The problem of compatibility between graft and receiver is real.

In the case of tissues, depending on the origin of the graft, we speak of autologous removal (done on the receiver himself) or heterologous removal (done on another person).

1.2.3. Organs donation in France

In France, organ and tissue donations are governed by bioethics laws. The three main principles are presumed consent, free donation and anonymity between donor and receiver. [5] The number of patients on the waiting list for organ and tissue transplants in France varies frequently.

Tissue transplants are increasing every year, but they are driven by the number of donors. However, the increase in the number of donors is not meeting the needs of the waiting receivers. [6] Concerning the skin, samples are progressing and are correlated with an increase in transplants: the number of tissue donors has stabilized and the surface area received in the bank has increased each year (Table 1). The more the quantity of skin received and available increases, the more the number of grafts increases, but the validated skin stocks are decreasing: the need remains high.

Skin	2013	2014	2015	2016	2017
Donation (m^2)	36.8	32.6	43.8	51.2	52.2
Graft (m^2)	29.6	24.6	33	45.4	49.1

Table 1: *Evolution of the surface of grafted skin in France from 2013 to 2017, Source: Annual activity report of French tissue banks.*

With so much strain on organ transplants and waiting lists, the value of organ bio-printing becomes evident. By overcoming ethical issues and compatibility between graft and receiver, printed organs and tissues are an interesting avenue for medicine.

1.3. Cosmetic tests

When a cosmetic product is developed, numerous preliminary tests are necessary in order to ensure health safety, to verify its possible toxicity and its stability. Careful consideration of the biological environment upon which it will exert its effects is also required. It is also possible to perform tests on the claims displayed (example: wrinkle reduction for an anti-wrinkle cream). [7]

Companies are responsible for placing their cosmetic products on the market and must ensure consumer safety: the product must not present any danger under normal conditions of use or under unusual but foreseeable conditions of use.

The safety assessment is conducted by a laboratory in most cases. First, conventional chemical tests are carried out in order to measure out the different components (heavy metals, polymers, etc.), then the cosmetics are tested on different skins. These come from live animals (outside Europe) or from paid volunteers.

While no shortage of volunteers or test animals is to be expected, these solutions pose obvious ethical problems. Samples of skin cultured by the conventional biological technique are taken, but these methods are expensive. 3D bio-printing is a serious and reliable alternative.

The conditions for marketing a cosmetic product on European soil are described in European Cosmetic Regulation n ° 1223/2009.

2. Bio-printing process

2.1. Skin background

Human skin is made up of several layers (see figure 1):

- The epidermis is the top layer of the skin, in direct contact with the environment. His thickness is about 0.1 millimeter. The surface of the skin is covered with dehydrated and hard cells: keratinocytes. These cells are attached to each other and offer very resistant protection against mechanical and chemical attacks. [8]

- The dermis contains blood vessels, ensures the innervation of the skin, and manages the production of sebum and sweat. It is made up of cells that produce collagen : fibroblasts. It is 5 times thicker than the epidermis.

- The hypodermis is the deepest layer. It is formed mainly of cells saturated with lipids which protect the subcutaneous structures against mechanical attacks and temperature variations.

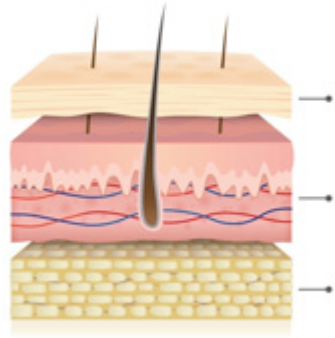

Figure 1: *Sectional diagram of the skin (source pileje.ch 2021)*

Skin grafting or transplantation is the use of a piece of skin to cover wounds and burns, either for the purpose of healing by the tissue provided or as a bandage. The skin is a regularly grafted tissue, especially in the case of third-degree burns. In most cases, this is an autologous transplant where the skin is simply removed from the patient.

The graft can be sewn, or glued with acrylic surgical glues or other glue types based on human protein (fibrin), sprayed under and over the graft. [9]

Tissue production can be carried out in two ways : from a stock of cells for large-scale industrial processes (allogeneic skin), or from the patient's own cells carried out case by case for therapeutic purposes (autologous skin). [10] For these two ways of production, it is preferable to have the best cell viability, which means the best ratio of alive cells during the grafting compared to the number of cells in total at the time of the transplant by the end of printing and fabric development.

2.2. Bio-printing processes

Two major principles exist in bio-printing. The first is the direct printing of cells from a bio ink with a high concentration of cells. This principle requires many precautions regarding to various stresses that the cells will undergo during printing (mechanical, thermal stresses, etc.). However, this approach has been shown to be successful by printing certain organs including cartilage prostheses [11].

The second principle is the impression of a nutrient medium in which cells will develop. Printing is followed by maturation in which the cells will divide and occupy the entire volume delimited by the nutrient medium. This principle is generally used for the skin.

Different printing techniques are used to make tissues and organs from a medium, however, the process always relies on similar steps: preparation, printing and maturation (analysis in Figure 2).

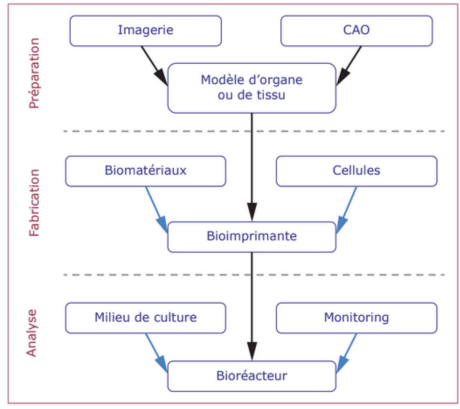

Figure 2: *Principle of bio-printing, Source: Engineering techniques, Guedon et Al., 2017*

1. Computer conceptualization of the architecture of the biological tissue then programming of the printing parameters of "bio inks" (cell suspensions). The cells are printed on support (hydrogel in the case of soft tissues) on which they will adhere and develop. Likewise, nutritional transfer processes must be anticipated to allow effective development of cells (example: vascularization);

2. The "biological tissues" are then printed layer by layer using machines that reproduce computer-designed patterns (see processes below);

3. The last step is based on the maturation of the printed fabric in a bioreactor. This step allows cells to self-organize and bind together in order to develop specific biological functions (liver, skin, etc.).

Once these steps are completed, it is possible to perform antigenic tests to verify the proper functioning of the cells.

2.3. Materials

2.3.1. Bio-ink

This specific ink is made up of cells and a matrix ("carrier") enveloping and protecting the cells. It is usually a biopolymer gel or protein matrix, which acts as a 3D molecular scaffold. The cells attach to the gel, allowing them to propagate, grow and proliferate. Bio-inks must exhibit certain characteristics, in particular rheological, mechanical, biofunctional and biocompatible properties.

It is important to note that the carrier offers considerable protection to the cells during the printing process. In the literature, the term "bio ink" is often used to describe the carrier alone.

Bio-inks have significant differences with conventional inks, in particular: printability at low temperatures (37 ° C or less), their crosslinking conditions are light, their natural origin, their bioactivity, and they must be rheofluidifiant.

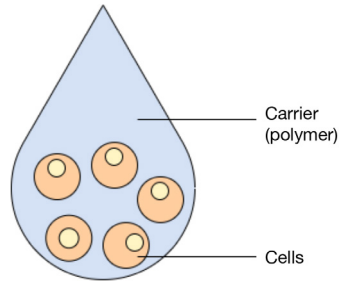

Figure 3: *Diagram of the bio ink Source: L. Pourchet. Development of a bio ink for 3D bio-printing of living tissue 2018*

2.3.2. Cells

Depending on the target organ, inks can have different cell compositions. In the case of skin creation, the target tissues are the epidermis and the dermis (the upper and middle layers of the skin, respectively). Therefore, the printed cells are keratinocytes and fibroblasts. Vascularization can be considered using vascular endothelial cells. It is also possible to add melanocytes to make the skin photosensitive and obtain coloration by the production of melanin after the skin is in operation.

Epidermal keratinocytes produce keratin, a protein that makes the skin strong. Dermal fibroblasts produce collagen, which is responsible for the elasticity of the skin. In order to test the viability of the cells, it is therefore, necessary to perform a keratin and collagen antigen test step which will give the rate of effective cells after the crosslinking step.

The necessary cells can be taken from a patient and then cultured for maximum compatibility (MHC) or cultured from laboratory cell lines. The cells are then isolated and separated before being put into solution in the carrier.

In the case of a skin impression, a suspension (in collagen) of 0.5 to 2 million cells / mL is used. Culture is 4 to 8 days before printing and then maturation (in the open air) for 10 to 14 days in order to obtain developed skin. [12]

2.3.3.Le carrier

The carrier is the matrix containing the cells in the bio ink. Several types of carriers exist.

The hydrogel is a matrix composed of two peptides (proteins): a hydrophobic gelling peptide and a hydrophilic surfactant. In an aqueous medium, the two peptides come together to create fibers and a structure that cells can attach to. The hydrophilic part of the hydrogel helps maintain a surface chemically suitable for cell development. The peptide surfactant can be tailored to target different uses and incorporate biological functionality (depending on the type of organ printed). The hydrogels are synthesized in the laboratory to ensure maximum reproducibility and stability. [13]

The carrier can also be in the form of a collagen-based protein matrix (used for skin creation). Collagen is a protein very present in mammals and in particular in tissues, it therefore has physicochemical properties adapted to them, as well as an in vitro / in vivo biocompatibility superior to synthetic matrices. This protein has been widely used in biomedical applications. In the case of a bio ink intended for the skin, the keratinocytes and fibroblasts can be encapsulated in a collagen matrix. [14]

The protein matrices can also be based on gelatin, but the results observed are less effective because of the lower mechanical strengths. [13]

2.4. 3D bio-printing existing processes

The term 3D bio-printing defines a set of printing techniques that can be grouped into several broad categories: laser, inkjet, extrusion and stereolithography (Table 2). There are also hybrid presses, combining several techniques.

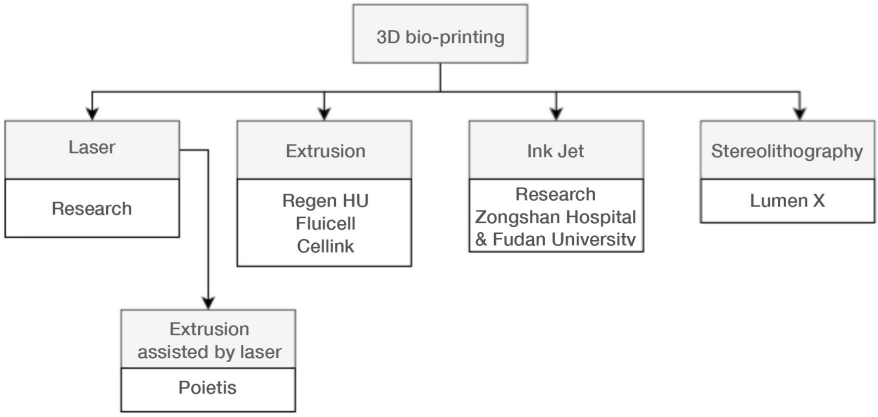

Table 2: *Main 3D bio-printing technologies and players, Source: S. Vijayavenkataraman et al. / Advanced Drug Delivery Reviews 132 (2018) 296–332, National University of Singapore, 2018*

The techniques used are very diverse and develop at different speeds. Several parameters must be taken into account when comparing techniques :

- Cell viability (or cell survival rate) is the ability of cells to survive and divide after the maturation phase;

- Scalability is the ability to adapt technology to human-sized organs and tissues;

- The resolution is often reduced to the size of the cells, the maximum resolution that can be achieved is that of a cell (about ten micrometers).

In this study, we will focus on two major processes in bio-printing research: inkjet and laser printing. We will briefly present the majority extrusion process in printers marketed today and the stereolithography process which is still in development.

2.5. Extrusion : industry's major process

Extrusion is the most widely used process. It is analogous to the conventional extrusion process. Bio ink is extruded out of a nozzle using pneumatic pressure or mechanical force by means of a plunger or screw. This process involves the printing of bone, cartilage, skeletal muscles, skin, heart tissue, nervous system, and liver in the field of bio-printing.

It has some advantages:

- Scalability, ie the possibility of creating tissues on a human scale (which is diffcult for other techniques)

- High viscosity of bio inks (≈ 600kPa.s) resulting in better protection of inks in the carrier

- A high concentration of cells

- Variable cell viability, 40-95% depending on the cells

However, it has limits:

- The resolution is very low (100µm) and does not allow the creation of complex tissues and makes any vascularization impossible;

- The clogging of the nozzles is very important (because the extruded fluid is very viscous);

- The inks must be rheo-fluidifying, which complicates the formulation and limits the range of bio inks that can be used.

It should be noted, however, that due to its ease of implementation and the volumes achievable, this technology has established itself in most printers on the market. It can be coupled with robotic arms, aiming lasers and microscopes to improve accuracy.

2.6. Stereolithography

The stereolithographic bio-printing process is similar to that used in "classic" 3D printing. It is a laser layer-by-layer polymerization of a soft, cross-linkable matrix. The excess material is then removed by rinsing. The structure formed is then matured in a bioreactor. The inks used have a very low viscosity (≈ 5Pa.s).

This technique has its advantages:

- High resolution ≈ 6 µm for conventional processes and up to ≈ 200 nm with dual-wavelength polymerization;

- The process is without a nozzle so there is no risk of clogging;

- The cells are not sheared.

But stereolithography also has its limits, which hamper the marketing of this technique:

- The bio inks used must contain a photoinitiator which is often cytotoxic and causes low viability of the cells (25%);

- Cells are exposed to UV during printing, although radiation is reflected and attenuated, UV remains a major factor in DNA degradation which can lead to cell death or dysfunction.

2.7. Historical process : inkjet

This process is an adaptation of the inkjet process for bio-printing by replacing the ink with a cell suspension.

2.7.1. Method

The inkjet printer works with a printhead that projects microdroplets of the bio ink containing cells. The formation of droplets is carried out by the instability of Raiylegh-Plateau and the ejection of the droplets is caused by a thermal (heat) or piezoelectric (electrical polarization of the ink under the action of mechanical stress) process. Bio ink is liquid at 20 ° C (printing temperature) but it gels at a temperature of 36 ° C (body temperature). This process is the most similar to that of plastic 3D printers.

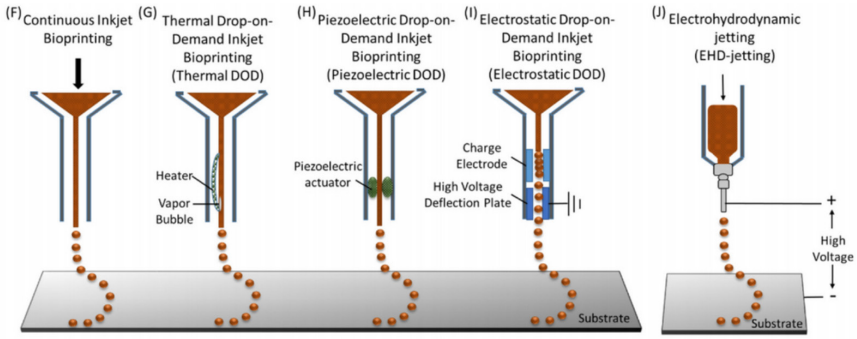

Figure 4 : *Diagram of the different inkjet printheads*

This process has been used to make tissues of : cartilage, skin, heart and nerve tissue.

2.7.2. Assets and disadvantages of inkjet

The advantages of inkjet are:

- High resolution can be achieved (≈ 50 μm);
- High printing speed (< 10,000 drops/sec);
- It is possible to introduce concentration gradients;
- Bio liquid inks (3 to 12 mPa.s);
- The process is inexpensive and requires little preparation.

The disadvantages of inkjet are:

- A step of cross-linking (a bond by covalent bridging between two molecules) is necessary to ensure the stability of the cellular structure;
- The viability of the cells is 80% to 85%;
- The presence of nozzles causes clogging problems and limits the concentration of organic inks at 106 cells/mL.

2.7.3. Perspectives

To solve the problem of cross-linking, an electrohydrodynamic inkjet (EHD-jetting) process has been developed. This involves applying a high voltage (0.5 to 20 kV) between the nozzle and the substrate to cause the drop to form in the nozzle. This process achieves very high resolutions, on the order of a nanometer, and significantly increases cell viability and avoids the cross-linking step. However, the inks must be very viscous, which poses plugging problems. This is a complex process to set up and very expensive.

While much research has been done on inkjet bio-printing, today few companies are commercializing the technology.

2.8. Evolving process : laser printing

2.8.1. Method

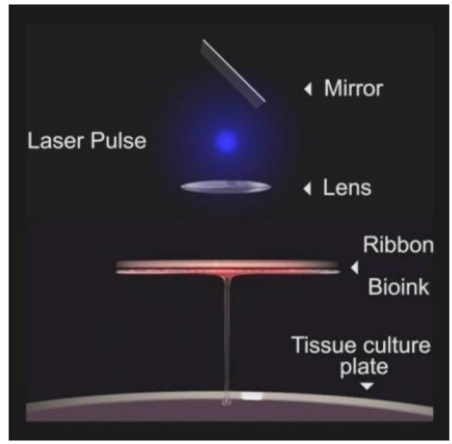

Figure 5: *Bio-printing assisted by laser graphic, INSERM 201*

The press is composed of an infrared laser source (continued or pulsed) reflected on a mirror to hit a laser transparent print ribbon on which the bio ink is coated. This is composed of cells. The substrate is placed under a moving plate, it may also be an inert matrix or a living tissue. In the case of a pulsed source, the projection capacity is approximately 10,000 micro-drops per second. The drops could achieve a size of a cell (about ≈ 20 µm), with an accuracy of a few microns. The layering of miro-dropplets allows to obtain a volume : it is a layer-by-layer digital printing.

Depending on the type of ribbon printing, different techniques are used. We won't go into details here.

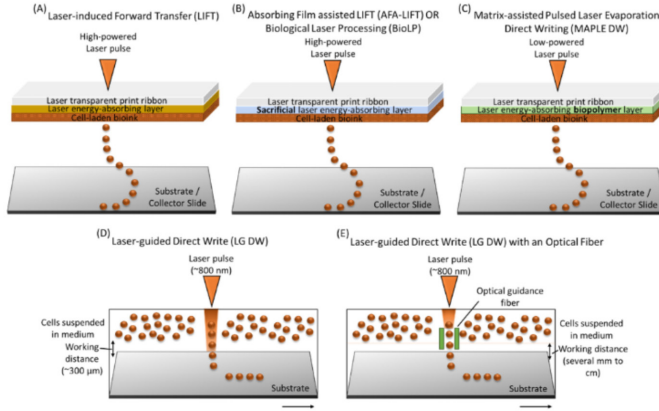

Figure 6: *Bio-printing principles schema, Source : 3D bio-printing of tissues and organs for regenerative medicine, Vijayavenkataraman et al, 2018*

2.8.2. Assets

Assets of laser bio-printing are :

- Contactless printing
- High viability of cells after printing (97% [INSERM 2015][15])
- The absence of a nozzle avoid clogging
- High resolution, ability to print 1 cell par drop (up to 108 cells/mL)
- Bio liquid ink (1 to 300 mPa.s)

2.8.3. Perspectives

Scientific literature report several tests concluding printing of fibroblasts, keratinocytes and dermal fibroblasts. In 2005, BIOTIS team of Bordeaux INSERM reached to print a multilayer of keratinocytes and collagen drawing near the skin.

Laser printing can be combined with extrusion to assure better scalability.

2.9. Summary table

	Ink Jet	Extrusion/Laser
Resolution	50 µm	20 µm
Printing speed	10,000 drops/s	10,000 drops/s
Ink viscosity	3 - 12 mPa.s	1 - 300 mPa.s
Cells viability	80 to 95 %	97 %
Financial resouces	low (compared to over process)	very high
Logistical means	low (compared to over process)	hardly industrialized
Disadvantages	Necessity of cross-linking	Need to master the conditions of polymerization, gelation and reticulation to preserve 3D structure [9]
Assets	Introduction of the compression gradient	Possibility to print 1 cell per drop

Table 3: *Comparative of the different printing processes*

The market of skin bio-printing is about the skin transplant but also the cosmetic market. It is interesting to study the market and make a competitive analysis.

3. Outlooks

3.1. Delimitation of the studied system

The economic situation of society is characterized by the statement, at one time, of economical and social variables on which it depend: production, the demand in service or in a product, investment, etc. [21]

3.1.1. Economic indicators

- Production variables

The cost and the production volumes are determined by twos factors : the cost and the availability of raw materials and 3D printers. Top range functional biological printers are very expensive (a few hundreds of thousands of euros) and could hardly be bought by hospitals or research laboratories. [22]

- Key technology variables

The mastery of the biological micro-environment, currently tough, could allow the complexification of the middle. The development of micro-vascularized tissues is the key element to succeed in the transition from in vitro bio-printed tissue in clinical development to transplantation. Indeed, is it possible to obtain a bigger tissue only if the structure is micro-vascularized. The improvement of printing speeds and maturation of cells could allow the optimization of the production of tissues and the yield.

3.1.2. Development factors

- Factors for social development

The interest of the general public : the public sees in bio-printing new methods of care and saving lives while reducing the disadvantages (like the need for removal of organs).

Ethical considerations rise by bio-printing are multiple. They could achieve in better case to favorise this solution faced to other (refusal animal testing) or rather the abandonment of this technology (refusal of artificial graft).

Inequality in access to this technology : bio-printing is a cutting-edge technology very expensive, and it will probably remain. It may not be accessible to everyone, this would create an inequality based on income and would accentuate the tendency whereby wealthier people have better cares and live longer.

- Factors of economical development

Public investment depends on the dynamism of the sector and the interest in this technology, as well as the economical situation of the concerned country.

Private investment depends on potential generated profits by using bio-printing.

- Legislative frame

State intervention in regulatory aspects of bio-printing is crucial to the future : too restrictive regulation which could lead to a black market of printed organs. In another way, a clear legislative frame in accordance with the needs would be a real asset for research and companies.

3.1.3. Major economic actors

- Public and private laboratories because they participate in the technical development of the organs created by print;

- Printer fabrication company;

- Country and their legislation

- Public hospitals, university-affiliated hospitals, private clinics could fund et run tests on the transplant;

- Major cosmetic or biological groups find interest in this technology and help to fund it.

3.2. Brakes on development

The request for tissue is very important, both in the reconstructive medicine field in the world of cosmetics, and research activity are widely supported by public and private actors. However, many roadblocks rise toward the democratization of this technology.

The first obstacle is the initial investment which is often really high. Printers, reagents, and stem cells reach a price toughly disbursing for laboratories and universities. The acquisition of certain stem cells line could also be complex.

In another way, a considerable legal effort is required to array this solution on the market. The different laws do not advance at the same pace. This factor could be a risk as well as an opportunity depending on the climate of the concerned country.

Finally, the last brake is technical : culture and incubation processes used after the printing are not suffciently advanced to form a bio printed tissue structure to

a completely functional organ, even if the skin is relatively easy to develop. This insecurity remains a high risk, particularly in the reliability and viability of tests or transplants carried out. [24]

4. Development of scenarios from coherent sets of hypotheses

Trend scenario: In 2040, Poietis holds 50% of the market share of bio-printing for skin grafts.

Hypotheses:

- The growth and interest in bio-printing continue to gain momentum.
- Less and less expensive technology
- Subsidy from major economic powers

Risk analysis for this scenario (ranked by risk importance):

- Legislation slowing down initiatives to test or graft printed skin, linked in particular to bioethical issues. (Probability of a slowdown of a few years: 50%)
- Technical impossibility of making the technology more affordable (scarcity of electronic components necessary for the manufacture of machines). (Probability of lack of raw resources : 20%)
- The appearance of a breakthrough technology renders bio-printing obsolete. (Probability of occurrence: 1%)

Scenario:
In 2017, a report by the firm SmarTech estimated the bio-printing market at $ 1.1 billion in 2027, up from $ 100 million in 2015, surpassing other markets related to 3D printing.

This growth can be explained by several factors, including the entry of multinational healthcare companies into the market. Other public actors, such as the European Union, which is already investing heavily in research projects on bio-printing, could continue to fund initiatives. Great powers like the United States and Canada will also increase their subsidies. The issue is recognized and will gain in importance in the coming years.

Thus, the growing number of research programs conducted on bio-printing could lead to the creation of more than 5,000 bio-printing systems around the world by 2027.

The most likely development is the accessibility of 3D bio-printing to a greater number of players within particular the appearance of less expensive systems, thus facilitating the dissemination of bio-printing in industry, but also universities.

In France, trials are already underway for the use of printed skin for cosmetic testing and skin grafts. These initiatives are closely related and will likely move forward quickly, provided the ethical issues raised by these technologies do not hinder their dissemination. It is reasonable to think that science will find a way to overcome these problems, for example by stopping the use of embryonic cells in favor of cells taken from the patient or from volunteers.

This is why a company like Poietis whose investment roughly doubles every year with an investment of 10 M€ in 2021 is surely set to grow significantly further before the arrival of potential competitors. What will stimulate the growth of this technology.

Negative scenario: In 2050, the discovery of new technology makes bio-printing obsolete

Hypotheses:

- Competition variable : Entry of a new more interesting competitor by simpler technical means, or more advantageous prices

- Technical variable : Encounter unexpected diffculties in the search for technical solutions

Risk analysis for this scenario (ranked by risk importance):
Although bio-printing is a very recent technology, it is already finding many limits. In fact, for the moment, knowledge about living things remains far too weak to equal the grafting of real skin and many techniques have not been mastered such as vascularization of the skin for example. There is no guarantee that these concepts will be fully discovered in the coming years because there is a whole field of life here that has never been studied until now. This scenario is imagined by numerous studies which recall the distance between the dream goal of bio-printing and reality [25]. The probability of having no improvement in knowledge and technique would be 5%.

These great times of implementation of this technology could also be accompanied by a new technology which would offer greater advantages than the current advantages of bio-printing, the probability of this happening would be 1% by observing the work of research carried out around the transplant in recent decades.

Scenario:
Currently, the main obstacle to the development of bio-printing is the lack of technical understanding of concepts of living things such as vascularization of the micro-environment. Current models of organs and tissues are not yet complete, and hardly take into account the complexity of an organ. However, they are the basis of 3D printing and their slow development is delaying the progress of bio-printing.

The slow development of 3D printing technologies can lead to a slowdown in innovation in the field. Bio-printing would then lose all its interest for lack of success. For example, it is possible never to end up with the vascularization of a printed bio system. The hypothesis according to which new, faster, more economical technologies will emerge in the coming years, rendering 3D printing obsolete, is also possible.

A company like Poietis raised 3 M€ in 2015, 5 M€ in 2017 and plans 10 M€ in 2021 [4]. The main investors in Poietis are public funding. If no satisfactory result does not emerge within the next few years, we can assume that interest in bio-printing will decline by 2050 leading to a decline in subsidies and research.

Positive scenario: In 2030, the bio-printing sector takes root in Asia

Variables:

- Legislative variable : Change in legislation allowing the opening of a new market

- Economic variable : private investment by companies in the cosmetics sector

Hypotheses:
China will ban cosmetic testing on animals in 2030. The cosmetics industry continues to grow at a steady pace.

Risk analysis for this scenario (ranked by risk importance) :

- Abandonment of tests on bio-printed skin in favor of another less expensive technology (probable, depends on the evolution of production variables and costs: probability of 40%)

- Legislation banning printed skin testing if the technology is not advanced enough by then. (unlikely because the printed skins are already reliable and used : probability of 5%)

- Loss of public interest in cosmetics. (very unlikely : probability of 1%)

In January 2021, China announced that it was considering dropping animal testing of certain cosmetic products imported to China provided the products meet health specifications. In 2024, China completely abandons the animal testing requirement and announces the ban on testing at the January 2030 limit to align with the European Union.

As a pillar of the Asian economy, China has a great influence on other Asian countries, like Taiwan, Thailand, the Philippines, Malaysia and South Korea, cosmetics consuming nations, could align with Chinese law, so as to create a favorable trading ground. Dermatological brands have achieved a penetration rate of almost 50% in Korea and Taiwan. The market is therefore constantly increasing.

If beauty product sales maintain their current popularity, the sector's growth rate may remain at an overall growth rate of + 5% over 10 years (2020-2030). The turnover of a company like L'Oréal could then reach 477 billion dollars and the R&D investment could reach 14 billion dollars in 2030. With their 15 years of experience in Europe, the major leading groups in cosmetics offer test solutions adapted to Asian legislation and invest heavily in bio-printing. Bio-printing is positioned as a reliable technology (skin replication is almost perfect), innovative (the image of the brand is preserved) and ecological (very little biological waste). The cosmetic testing market for Asia is opening up for bio-Impression.

Conclusion

Bio-printing is still in its infancy and a lot of work is necessary in order to properly carry out this technology to industrialization. One of these processes which seems to be the most affordable is skin printing.

The actual state of the art allows the realization of projects at a small scale, such as the use of printed skin for cosmetics that's why it is interesting to optimize the printing process and search for a bio ink with better properties. Nowadays, developed techniques (extrusion, ink jet and laser) are too coarse to allow complex organs printing, but are adapted to skin tissue printing. Stereolithography could allow a better accuracy, but output volumes are insuffcient at the moment.

That's why it is necessary to stay alert of the real possibilities that offer bio-printing in the skin field. The issue is recognized by the biggest cosmetic groups of the world, leading universities and the bigger decision-maker such as Europe and the FDA in the USA.

One of the waiting development in a near future is tissue vascularization to permit the creation of a blood network in printed tissues.

Bibliographie

[1]: Wikipedia. (consultée le 14/03/2021). Impression 3D. [en ligne]. https://fr.wikipedia.org/wiki/Impression_3D

[2]: Wikipedia. (consultée le 14/03/2021). Bio-Impression. [en ligne]. https://fr.wikipedia.org/wiki/Bio- impression#Historique

[3]: Guédon E, Malaquin L, André JC. Bio-printing - Etat des lieux et perspectives. Technique de l'ingénieur. 2017 Feb 10 p.17

[4]: INSERM. (consultée le 14/03/2021). Transplantation d'organes / Greffes. [en ligne]. https://www.inserm.fr/information-en-sante/dossiers-information/transplantation-organes-greffe

[5]: Agence de la Biomédecine. (consultée le 13/03/2021). Que dit la loi sur le don d'organes et de tissus ? [en ligne] https://www.dondorganes.fr

[6]: Agence de la biomédecine. (consultée le 14/03/2021). Le rapport médical et scientifique de la biomédecine. [en ligne]. https://www.agence-biomedecine.fr/annexes/bilan2017/donnees/prelevement/01-tissus/synthese.htm

[7]: Pôle Cosmétique. (consultée le 20/05/2021). Les tests d'évaluation cosmétique. [En ligne]. https://pole-cosmetique.fr/faq/les-tests-d-evaluation-cosmetique/

[8]: Medi-France. (consultée le 14/03/2021). La peau, un organe multifonction qui a des exigences. [en ligne]. https://www.medi-france.com/sante/le-corps-humain/peau/

[9]: R. Alvarez, M.-H. Tarteaut, M. Szewczyk, S. Marcionetti, K. Jaggi, G.-N. Donnat L. Blal. (consultée le 14/03/2021). Soin suite à une greffe de peau mince. [En ligne]. https://www.hug.ch/procedures-de-soins/soins-suite-une-greffe-de- peau-mince

[10]: Universidad Carlos III de Madrid - Oficina de Información Científica. (consultée le 14/03/2021). 3-D bioprinter to print human skin [En ligne]. https://www.sciencedaily.com/releases/2017/01/170123090630.htm

[11]: Pourchet L. Développement d'une bio-encre pour la bioimpression 3D de tissus vivants : étude de la formulation et caractérisation du développement tissulaire. Biotechnologie. Université de Lyon, 2018. Français. ffNNT: 2018LYSE1232ff. fftel-02065562f p.196

[12]: Pourchet L. Développement d'une bio-encre pour la bioimpression 3D de tissus vivants : étude de la formulation et caractérisation du développement tissulaire. Biotechnologie. Université de Lyon, 2018. Français. ffNNT: 2018LYSE1232ff. fftel-02065562f p.44

[13]: Biogelx. (consultée le 05/03/2021). Hydrogel for 3D cell culture and bio-printing. [en ligne]. https:// www.biogelx.com

[14]: Gunger-Oskerim PS, Inci I, Zang YS, Kademhosseini A, Dokmeci MR. (consultée le 14/03/2021). Bioinks for 3D bio-printing : an overview. [En ligne]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6439477/

[15]: Medi-France. (consultée le 14/03/2021). La peau, un organe multifonction qui a des exigences. [en ligne]. https://www.medi-france.com/sante/le-corps-humain/peau/

[16]: Rapport annuel L'Oréal 2019, consulté le 09/04/21, site: https://www.loreal-finance.com/fr/rapport-annuel-2019/marche-cosmetique-2-1-0/

[17]: Inneance. (consultée le 06/05/2021) Bio impression et médecine régénérative: où on en est ? [en ligne]vhttps://www.inneance.fr/bio-impression-et-medecine-regenerative-ou-en-est-on/

[18]: Wikipedia. (consultée le 06/05/2021) Bio impression/ interdiction. [en ligne] https://fr.wikipedia.org/wiki/Bio-impression#Interdiction

[19]: Agence Biomédecine. (Consulté le 25/04/2021). La rapport médical et scientifique de la biomédecine 2017. [en ligne]. https://www.agence-biomedecine.fr/annexes/bilan2017/donnees/prelevement/01-tissus/synthese.htm

[20]: France Adot. (consultée le 25/04/2021). La législation en matière de don d'organes [En ligne]. https://www.france-adot.org/la-legislation-en-matiere-de-don-dorganes/

[21]: Rapport annuel L'Oréal 2019, consulté le 09/04/21, site: https://www.loreal-finance.com/fr/rapport-annuel-2019/marche-cosmetique-2-1-0/

[22]: Interdiction de l'expérimentation animale, Fédération des entreprises de la beauté, 31/03/2020. Consulté le 20/04/2021, site : https://www.febea.fr/fr/interdiction-lexperimentation-animale

[23]: Article, Fédération des entreprises de la beauté. Consulté le 20/04/2021, site : https://www.febea.fr/fr/vos-produits- cosmetiques/actualites/2021-la-chine-va-franchir-etape-decisive-larret-tests-animaux

[24]: Aniwaa. (consultée le 25/04/2021). Bio-Impression 3D : guide et sélection d'imprimante 3D. [En ligne]. https://www.aniwaa.fr/guide-achat/imprimantes-3d/bio-impression-3d-bio-imprimantes/#Mentions_speciales_et_alternat ives

[25]: Guédon E, Malaquin L, André JC. Bio-printing - Etat des lieux et perspectives. Technique de l'ingénieur. 2017 Feb 10 p.18