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## Bioengineered Skin: The Self-Assembly Approach

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### Abstract

Tissue-engineered skin substitutes represent an innovative therapeutic option for the treatment of burns and skin ulcers as well as a powerful tool for fundamental research. To be efficient, *in vitro* skin substitutes must closely mimic human skin structures and exogenous material has to be reduced as much as possible. The self-assembly approach is based on the capacity of fibroblasts to create their own extracellular matrix *in vitro*, which allows the production of cell sheets that are easy to handle. Therefore, a skin substitute devoid of exogenous extracellular matrix proteins and synthetic material is produced, which demonstrates many histological, physico-chemical and mechanical characteristics found in normal human skin *in vivo*. A particularity of this approach is the possibility to add various other cell types (keratinocytes, melanocytes, adipocytes, endothelial and immunological cells, etc.) according to needs. Furthermore, pathological cells (hypertrophic scar, sclerodermic, tumoral and psoriatic cells) can be used for the production of pathological skin substitutes. The development of these models represents a key component in the fight against such diseases because they can lead to a better understanding of the pathology and to the development of new pharmaceutical therapies. This review will present the need for tissue-engineered skin substitutes, the implication of tissue engineering in the cutaneous field (basic and applied research), the self-assembly approach and its characteristics as well as the actual state of research on healthy and pathological self-assembled skin models.

**Keywords:** Tissue engineering; Skin substitutes; Self-assembly approach

### Introduction

Diseases, congenital defects or trauma can impair organ and tissue functions [1]. These damages affect individual physical and psychological well-being and cause health problems. Replacement, repair or regeneration of the impaired organ is a way to counter this situation [1]. Since the 50s, improvements in organ transplantation, such as the appearance of innovative surgical techniques, new supply sources and the development of immunosuppressive treatments, have contributed to the success ratio of modern transplant. In 1954, Dr. Joseph Murray performed the first successful living-related kidney transplant between two identical twins. The next organs to be successfully transplanted were the lungs in 1963 by Dr. James Hardy followed by the pancreas (1966), liver (1967), heart (1967), hand (1998) and full face (2010) transplants [2]. In the United States, more than 475 000 organ transplants have been done in the last 23 years. More than 28 000 of those transplants occurred in 2009, when nearly 15 000 people donated organs [3]. Now, organ transplants, autograft and prosthetic implants are commonly used to replace or to repair an organ. However, the need for donated organs greatly exceeds their availability, organ waiting lists are growing, and patients die expecting available organs [3].

### The need for tissue-engineered substitutes

Recent biotechnological progress suggests that tissue engineering is an interesting approach to counteract organ deficit [4]. Effectively, tissue-engineered techniques allow to produce, *in vitro*, a large variety of organs and tissues (cartilage, cornea, blood vessels, skin, etc.) for a broad spectrum of applications: orthopaedic, pneumologic, ophthalmologic, urologic, neurologic, vascular, stem cells and dermatologic. Tissue engineering increasingly focuses on the development of biological substitutes, in order to restore, maintain or repair the tissue functions [5,6]. It can also help to improve our understanding of the interactions and structure of healthy and pathological tissues [7]. Tissue engineering

encompasses a multitude of techniques including biomaterials, cells, growth factors and engineering components such as pumps, tubes, bioreactors and oxygenators [8]. Cells used in the production of tissues can be of diverse origins (xenogeneic, allogeneic or autogeneic). The production of autologous substitutes, from a patient's own cells, circumvents the risk of disease transmission between donor and receiver. Furthermore, autologous substitutes also prevent rejection of the graft with no need for immunosuppressive therapy.

### Tissue engineering vs. cutaneous field

Tissue-engineered skin substitutes represent an innovative therapeutic option for the treatment of burns and skin ulcers as well as a powerful tool for fundamental research [7,9]. In fact, they can be used as a replacement for human skin in clinical applications and in various fields of fundamental research such as skin biology, pharmacotoxicology as well as cellular and molecular biology [7,9]. Reconstructed skin substitutes produced by tissue engineering are mostly used for the treatment of burn victims [10]. Each year, more than 2 million of American patients need medical care following burns. Among them, 20 000 will suffer severe burns and will necessitate hospital admission in a burn unit [9]. Available skin donor sites may be very limited in extensive burn patients and simple autografting techniques are often not possible. Various commercially available products can

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provide temporary wound cover. However, only those that incorporate living autologous keratinocytes and epidermal stem cells can lead to the formation of a permanent, self-sustaining epidermis. This has fostered the development of alternative approach such as skin substitutes [9]. Effectively, from a small biopsy of skin, tissue engineering allows the production of many skin substitutes which can also be used in the treatment of skin ulcers. Diabetic ulcers affect 4.4% to 10.5% of diabetic patients, resulting in 82 000 lower extremity amputations each year; venous leg ulcers touch 0.2% to 1.4% of the world population whereas the prevalence of pressure ulcers is estimated to 5.0% to 8.8% of institutionalized patients [8]. Skin substitutes are an interesting way to counter these problems.

Such as previously mentioned, another considerable aspect of tissue engineering is its usefulness for fundamental research. *In vitro* production of skin substitutes may be useful to test new treatments or to better understand cell mechanisms (cell proliferation and differentiation, inflammation, intercellular interactions, etc.) [11]. Normal skin substitutes are mainly used for dermopharmaceutical, pharmacotoxicological and, sometimes, for physiological assays [12]. A broad variety of normal skin substitutes are commercially available for clinical applications and fundamental research. Each type of substitutes has its advantages and inconveniences. An exhaustive list of substitutes currently available on the market is presented in **Table 1** including clinical applications and fundamental research references.

Commercial product	Description	References
<b>Epidermal substitutes</b>		
BioSeed-S™	Subconfluent autologous keratinocytes on a fibrin matrix	[14,15]
CellSpray™	Noncultured autologous keratinocyte suspension	-
Cryoskin™	Cryopreserved monolayer of noncultured allogeneic keratinocytes coating with silicone backing	[16,17]
Epibase	Cultured autologous keratinocytes	[18,21]
Epicel®	Cultured autologous keratinocytes from skin on petrolatum gauze backing	[8,22,23]
Epidex™	Cultured autologous keratinocytes from the outer root sheath on silicone membrane	[24-26]
Episkin™	Cultured keratinocytes on a collagen matrix	[27-29]
Laserskin™ (Vivoderm™)	Cultured autologous keratinocytes in a matrix of a hyaluronic acid ester	[30-37]
LyphoDerm™	Freeze-dried lysate from cultured allogeneic epidermal keratinocytes into a hydrophilic gel	[38]
Myskin™	Cultured autologous keratinocytes seeded on specially treated silicone sheet	[39,40]
ReCell®	Noncultured autologous keratinocyte suspension	[41-45]
Suprathel®	Absorbable, synthetic wound dressing with properties of natural epithelium	[46-48]
<b>Dermal substitutes</b>		
AlloDerm™	Allogeneic acellular dermal matrix	[49-54]
Biobrane™	Porcine collagen chemically bound to silicone/nylon membrane	[55-58]
Cymetra™	Micronized particulate acellular cadaveric dermal matrix	[59]
Dermagen	Allogeneic fibroblasts cultured in a collagenous sponge	[60]
Dermagraft™	Allogeneic living human-derived fibroblast skin substitute	[54,61-63]
Dermamatrix	Allogeneic acellular human dermis	[49,50]
EZ-Derm™	Acellular xenogeneic collagen matrix	[64,65]
FortaFlex™	Acellular collagen matrix material derived from porcine small intestine submucosa	-
Glyaderm®	Acellular human dermis	[66,67]
GraftJacket®	Allogeneic human acellular pre-meshed dermis	[68-70]
Hyalograft 3D™	Autologous dermal substitute including a matrix of a hyaluronic acid ester	[30,31,71,72]
ICX-SKN	Allogeneic dermal substitute with human dermal fibroblasts in human collagen matrix	[73,74]
Integra®	Nonliving extracellular matrix of collagen and chondroitin-6-sulfate with silicone backing	[45,75-82]
Karoderm	Allogeneic human acellular dermis	-
Matriderm™	Acellular scaffold composed of elastin and collagen types I, III and V	[83-88]
Oasis™	Acellular collagen matrix material derived porcine small intestinal submucosa	[89-91]
Permacol Surgical Implant	Acellular porcine dermis	[92-94]
Repliform™	Acellular cadaveric human dermal allograft	[95]
Strattice™	Acellular porcine dermis	[96-99]
SureDerm	Allogeneic acellular human lyophilized dermis	[100,101]
TransCyte™	Polymer membrane and allogeneic neonatal human fibroblast cells on a nylon mesh coated with porcine dermal collagen and bonded to a polymer membrane (silicone)	[102-106]
<b>Bilayer substitutes</b>		
Apligraf®	Allogeneic cultured human keratinocytes and fibroblasts in a bovine collagen sponge	[107-109]
OrCel®	Similar to Apligraf®	[110,111]
PermaDerm™	Autologous keratinocytes seeded onto dermal substitute made with autologous fibroblasts in bovine collagen matrix	[112,113]
PolyActive	Autologous cultured keratinocytes and fibroblasts in elastomeric and biodegradable polyethylene oxide terephthalate/polybutylene terephthalate copolymer	[114,117]
StrataGraft®	Allogeneic dermis and epidermis generated from a progenitor cell line: neonatal immortalized keratinocytes (NIKS®)	[118,119]
TissueTech™	Autologous dermal substitute Hyalograft 3D combined with an autologous epidermal replacement (Laserskin autograft)	[31,120]

**Table 1 :** Commercially available skin substitutes.

Skin substitutes can also be pathological. *In vitro* pathological models are a good alternative to animal models. These models are produced with pathological cells, in order to mimic the main features of the pathology. Their main functions include: (1) testing new drugs and (2) performing pathophysiological studies in order to better understand the underlying mechanisms of the pathology. However, many of these models do not mimic closely enough the pathological characteristics of interest and researchers stay alert for the development of more relevant pathological models [13].

### Self-assembly approach

To be effective, *in vitro* skin substitutes must closely mimic human skin structure [121] and exogenous materials has to be reduced as much as possible. The self-assembly approach allows the production of skin substitutes devoid of exogenous extracellular matrix proteins and synthetic materials that also demonstrate many histological, physico-chemical and mechanical characteristics found in normal human skin *in vivo* [13].

### Self-assembly approach methodology

In the self-assembly approach, fibroblasts are cultured in the presence of ascorbic acid (50 µg/mL) for 4 weeks, thus forming manipulatable sheets, which are superimposed and incubated for 7 days to form the dermal component. After 7 days of culture, keratinocytes can be seeded upon this tissue to form the epidermal layer. After another 7 days of culture, the substitutes are raised to the air-liquid interface [122,123]. Biopsies are taken after 7, 14 and/or 21 days of culture at the air-liquid interface and analyzed using histological, immunohistochemical, permeability or physico-chemical techniques (Figure 1).

### Self-assembly approach characteristics

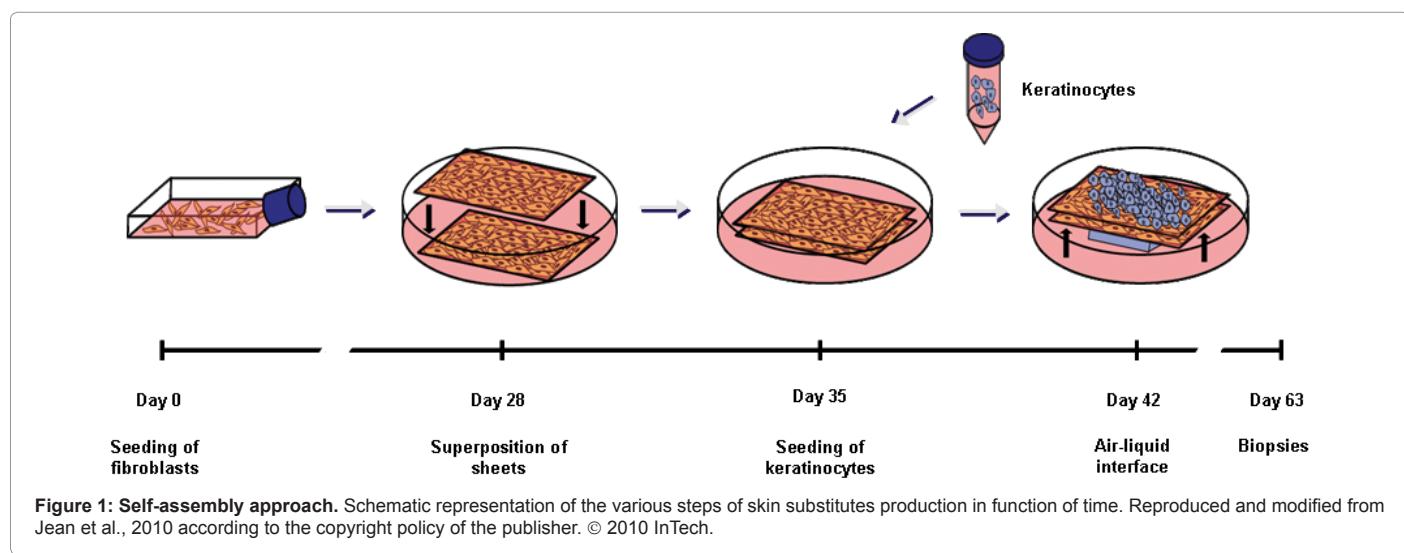
As previously reported, substitutes produced with the self-assembly approach share many characteristics with normal human skin. These features will be discussed in this section.

First, self-assembled skin substitutes possess many histological characteristics close to those observed in normal human skin, such as the presence of both dermis and epidermis. The reconstructed epidermis is well-differentiated, including the presence of all epidermal layers of the

native tissue: *stratum basale*, *stratum spinosum*, *stratum granulosum* and *stratum corneum* (Figure 2) [122,123]. The reconstructed dermis is well-colonized by the fibroblasts, which synthesize an abundant extracellular matrix [7]. Epidermal thickness varies in function of culture time whereas the dermal thickness differs according to the number of superimposed fibroblast sheets used (see Section of the self-assembly approach).

Second, expression (and localization) data for many proliferation and differentiation markers, as well as dermo-epidermal and extracellular markers, revealed that skin substitutes produced with the self-assembly approach share common structures with normal human skin (Table 2) [124]. Effectively, in both normal human skin and self-assembled skin substitutes, the expression of involucrin appears in the late spinous layer of the epidermis whereas loricrin is expressed in the granular layer (Figure 3A) [11]. Collagen I and III, two constituents of the extracellular matrix, are expressed in a similar way in the dermis of normal human skin and in the self-assembled skin substitutes (Figure 3B) [11]. Finally, markers of the dermo-epidermal junction, such as laminin V and human collagen type VII, are expressed in the dermo-epidermal junction of self-assembled substitutes such as what can be observed in normal human skin (Figure 4) [11].

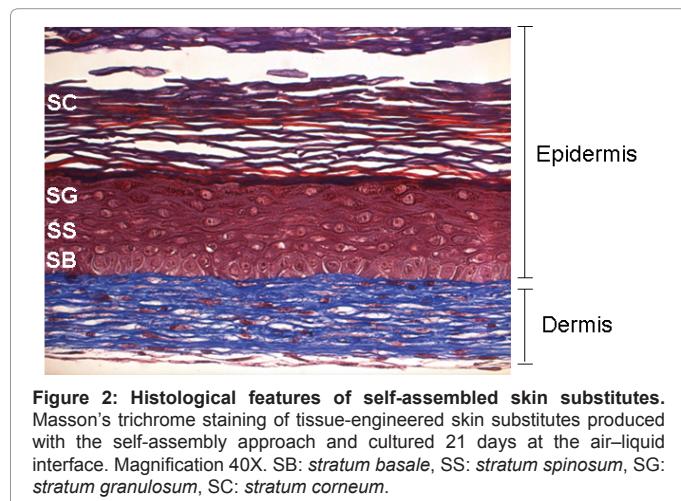
Third, *stratum corneum* is the primary protective barrier of the organism against chemical and biological external agents as well as water loss [125]. Well-structured lipid organization and impermeability of the *stratum corneum* are two essential criteria to obtain valuable tissue-engineered skin substitutes. Previous study using ATR-FTIR techniques had demonstrated that self-assembled skin substitutes possess an efficient lipid organization in the *stratum corneum*, much better than that observed in collagen gel skin substitutes. Effectively, self-assembled skin substitutes show lower frequencies in CH<sub>2</sub> bands compared with collagen gel substitutes and higher frequencies when compared with normal human skin (Figure 5) [4]. Higher frequencies in CH<sub>2</sub> bands are generally characteristic of high content of gauche conformers while bands at lower frequencies are associated to the presence of trans conformers. The variation of frequencies in function of the temperature shows a phase transition from an ordered system to a disordered conformation [11]. The results generated by the study of lipid organization in the *stratum corneum* can be summarized as follows: collagen gel substitutes < self-assembled skin substitutes < normal human skin.



Markers	Expression	
	Human skin	Skin substitute
<b>Epidermal</b>		
Keratins 5 and 14	SB	SB; SSB
Keratins 1 and 10	SSB	SSB
Involucrin	SP; SG	SP; SG (Figure 3A)
Transglutaminase	SP; SG	SP; SG
Loricrin	SG	SP; SG (Figure 3A)
Filaggrin	SG	SP; SG
DLK	SG	SG
<b>Dermo-epidermal</b>		
Laminin V	BM	BM (Figure 4)
Collagen IV	BM	BM; dermis
Collagen VII	BM	BM (Figure 4)
<b>Dermal</b>		
Collagen I	Dermis	Dermis (Figure 3B)
Collagen III	Dermis	Dermis (Figure 3B)
Fibronectin	Dermis	Dermis

SB: stratum basale; SP: stratum spinosum; SG: stratum granulosum; SC: stratum corneum; SSB: stratum suprabasale; BM: basement membrane

**Table 2:** Expression of epidermal, dermo-epidermal and dermal markers in human skin and in skin substitute produced with the self-assembly approach.



**Figure 2: Histological features of self-assembled skin substitutes.** Masson's trichrome staining of tissue-engineered skin substitutes produced with the self-assembly approach and cultured 21 days at the air-liquid interface. Magnification 40X. SB: stratum basale, SS: stratum spinosum, SG: stratum granulosum, SC: stratum corneum.

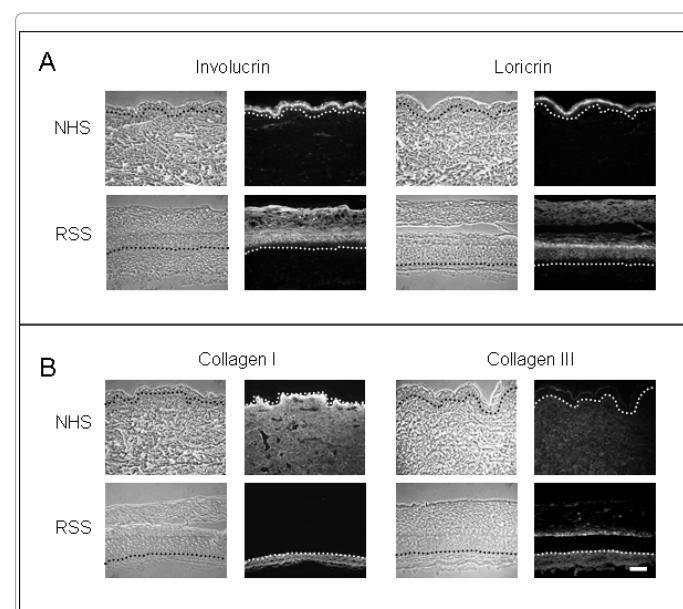
Finally, permeability results obtained by percutaneous absorption analyses demonstrate that self-assembled skin substitutes possess an impressive impermeability profile, close to that observed in normal human skin, as measured with a lipophilic molecule such as hydrocortisone (Figure 6) [11].

### Self-assembly approach for clinical use and fundamental research

Normal skin substitutes can be used to cover burns, skin ulcers and also serve as *in vitro* models in various fields of fundamental research including skin biology, pharmacotoxicology as well as in cell and molecular biology studies [7,9,10]. Normal skin substitutes are produced with autologous normal cells. On the other hand, pathological skin substitutes can also be produced with the self-assembly approach by using pathological cells of various skin diseases. These substitutes are not used as a replacement for clinical applications, but as pathological models for preclinical studies, to better understand mechanisms of diseases and to observe the effects of new treatments. Pathological self-assembled skin substitutes display some cell-specific

pathological characteristics of the affected tissues from which the cells were extracted, such as hypertrophic scars, psoriasis and scleroderma [13,126,127]. For example, in 2009, Jean *et al.*, demonstrated that psoriatic substitutes produced with the self-assembly approach maintained many characteristics of the disease including the presence of a disorganized and thicker epidermis compared with normal skin substitutes. These observations suggest the presence of keratinocyte hyperproliferation and abnormal differentiation that characterize psoriatic skin *in vivo* (Figure 7) [13]. So far, many cell types are used for the production of self-assembled skin substitutes and three skin diseases can be partially reproduced. A list of normal and pathological cell types used for the production of self-assembled substitutes is presented in Table 3.

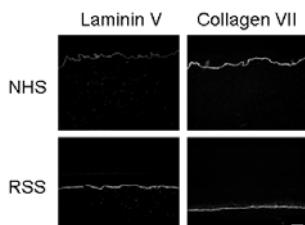
Overall, the self-assembly approach allows the understanding of pathological skin complexity through the possibility of: (1) dissecting step by step the mechanisms of skin pathologies according to which kind of cells is present in the model at this time and/or (2) using various cell combinations such as healthy fibroblasts and healthy keratinocytes, which can be compared with healthy fibroblasts and pathological



**Figure 3: Epidermal and dermal immunohistochemical analyses of skin substitutes.** Immunolabelling of epidermal differentiation markers (A) and extracellular matrix (B) of substitutes produced with the self-assembly approach. NHS: normal human skin; RSS: reconstructed skin substitute (scale bar = 50 µm). The dotted line indicates the dermo-epidermal junction. Reproduced and modified from Jean *et al.*, 2011 according to the copyright policy of the publisher. © 2011 Mary Ann Liebert, Inc.

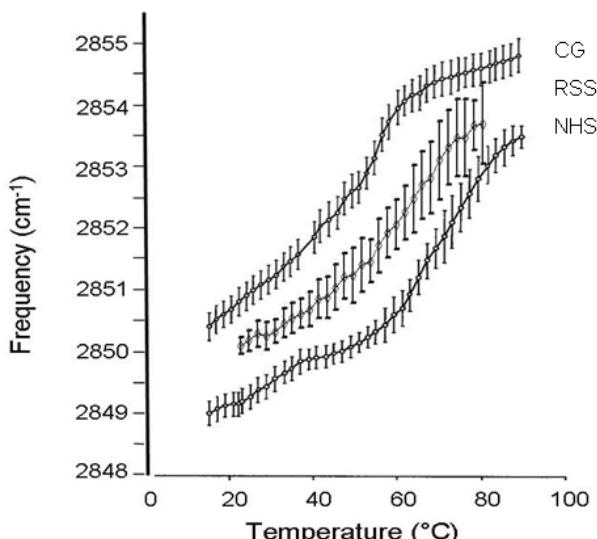
Cell types	References
<b>Normal cells</b>	
Adipocyte	[128-131]
Endothelial	[132]
Melanocyte	-
Stem	[133-136]
<b>Pathological cells</b>	
Hypertrophic scar	[126]
Psoriatic	[4,13,137]
Sclerodermic	[127]

**Table 3:** Normal and pathological cell types used for the production of skin substitutes produced with the self-assembly approach.



**Figure 4: Immunohistochemical analyses of the dermo-epidermal junction of skin substitutes.** Immunolabelling of the dermo-epidermal junction: laminin V and collagen VII of skin substitutes produced with the self-assembly approach. NHS: normal human skin; RSS: reconstructed skin substitute (scale bar = 50 µm). Reproduced and modified from Jean et al., 2011 according to the copyright policy of the publisher. © 2011 Mary Ann Liebert, Inc.

[114-117] matrix. As for the StrataGraft® model, it is produced with allogeneic dermis and epidermis generated from a progenitor cell line: neonatal immortalized keratinocytes (NIKS®) cultured in a non-bovine source of purified type I collagen [118,119]. Although only Apligraft®, OrCel® and StrataGraft® use allogeneic keratinocytes and fibroblasts, all six commercially bilayer skin models are characterized by the use of exogenous material to produce the dermal matrix. Self-assembled reconstructed skins share some features with commercially available bilayer skin substitutes. However, they set themselves apart from the others because: (1) they are produced with autologous cells and (2) they are completely free of exogenous biomaterials, thus eliminating risks of rejection, infection or immune reactions [7]. An exogenous-free materials are clearly advantageous for clinical applications and laboratory research, but its production is time-consuming. Indeed, the production of a complete autologous extracellular matrix necessitates

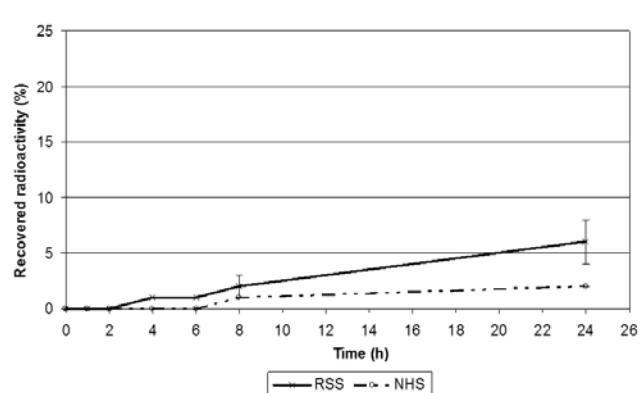


**Figure 5: Comparison of the stratum corneum lipid organization of collagen gel substitute, self-assembled skin substitute and normal human skin.** ATR-FTIR spectroscopy results obtained from collagen gel skin substitute (CG), self-assembled skin substitutes (RSS) and normal human skin (NHS). Reproduced and modified from Bernard et al., 2007 according to the copyright policy of the publisher. © 2007 Elsevier.

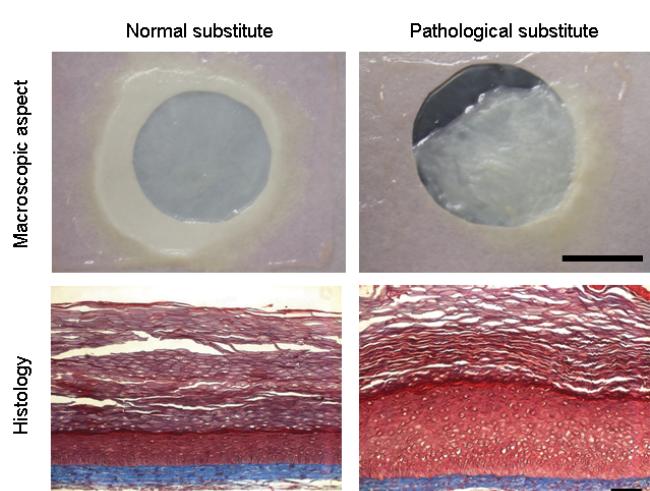
keratinocytes to analyse the interaction between different cell types [138]. These combinations are not observed *in vivo* and could only be studied with *in vitro* skin models.

#### The self-assembly approach compared with other skin substitute models

Commercially available skin substitutes are grouped into three categories: epidermal, dermal and bilayer skin substitutes. Reconstructed skin substitutes produced with the self-assembly approach can be included in the bilayer category, because they are composed of both dermis and epidermis. This category includes six models characterized by the presence of autologous or allogeneic cells cultured with exogenous material. In fact, Apligraft® and OrCel® are produced with allogeneic human keratinocytes and fibroblasts, cultured in a bovine collagen sponge [107-111] whereas other bilayer skin models are produced with autologous keratinocytes, seeded onto a dermal substitute made with autologous fibroblasts in a bovine collagen (PermaDerm™) [112,113], hyaluronic acid ester (TissueTech™) [31,120] or polymer (Polyactive)



**Figure 6: Comparison of the percutaneous absorption of self-assembled skin substitute and normal human skin.** Hydrocortisone penetration profiles through skin of self-assembled skin substitute and normal human skin. NHS: normal human skin; RSS: reconstructed skin substitute. Reproduced and modified from Jean et al., 2011 according to the copyright policy of the publisher. © 2011 Mary Ann Liebert, Inc.



**Figure 7: Macroscopic appearance and histological analysis of normal and pathological skin substitutes.** Macroscopic and histological analyses of normal and pathological skin substitutes after 21 days of culture at the air-liquid interface (scale bars = 2.2 cm and 50 µm, respectively). Reproduced and modified from Jean et al., 2009 according to the copyright policy of the publisher. 2009 Elsevier.

three months of culture. Thus, substitutes produced with the self-assembly approach can be used for clinical applications if the situation allows it; however, in emergency applications where time is of the essence, commercially skin substitutes can be a good alternative.

### The self-assembly approach used in other tissue-engineered fields

This review highlighted the production of skin substitutes with the self-assembly approach. However, it is important to mention that the self-assembly approach can be used in various fields of tissue engineering. Since 1990s, it has also been adapted for the production of reconstructed blood vessels, bladder and cornea [139,140]. In 1998, L'heureux *et al.* published data on reconstructed blood vessels produced with the self-assembly approach, which demonstrated a functional endothelium and positively reacted to surgical handling. It is important to note that this team was the first to obtain a completely biological tissue-engineered blood vessel with a burst strength comparable to what is observed in human blood vessels [139].

In 2006, Magnan *et al.* developed an endothelialized bladder from a single porcine biopsy [140]. They demonstrated that reconstructed bladder substitutes that possessed a thickness of at least 2 mm, were able to promote the formation of capillary-like tubes [140] and presented an efficient permeability barrier to urea [141]. Following this accomplishment, they studied the possibility to produce urinary tracts with the self-assembly approach [142]. Interesting results were obtained regarding the possibility to test this reconstructed bladder on bigger animals [143].

Another possible application of the self-assembly approach is the production of corneas with autologous cells. The current state of knowledge shows that it is possible to produce a complete autologous tissue-engineered human cornea, similar to native corneas, using untransformed fibroblasts, epithelial and endothelial cells [144]. The self-assembly approach has already opened new horizons and the transfer of this technique to other tissue-engineering applications is probable in the near future.

## Conclusion

Skin substitutes play a crucial role in both clinical applications and fundamental research. They can provide a mechanical barrier against infection and fluid loss [10] or replace animal models for dermopharmaceutical testings [4,11]. Skin substitutes can also lead to a better understanding of pathological and/or normal skin mechanisms [11]. There is a large variety of substitutes in the “skin substitute family”, commercially available or not, and the self-assembled model is one member of this family.

### The self-assembly approach benefits

Since 1990s, the self-assembly approach has been developed for the production of a broad variety of tissue-engineered substitutes (reconstructed skin, blood vessels, bladder and cornea). This approach has demonstrated many advantages compared to other models in both clinical applications and fundamental research.

### Clinical application benefits

The self-assembly approach allows the production of autologous skin substitutes devoid of exogenous material. The production of autologous substitutes, from a patient's own cells, circumvents the risk of disease transmission between donor and receiver and also

prevents graft rejection without the need for immunosuppressive therapy. Moreover, the absence of exogenous material eliminates risks of infection or immune reactions for patients following skin graft [7]. From a small skin biopsy, thousand of self-assembled skin substitutes can be produced. These substitutes are useful to cover a variety of large scale wounds (burns or diabetic ulcers). Finally, the major interest of the self-assembled skin substitutes is the incorporation of living autologous keratinocytes and epidermal stem cells, which can lead to the formation of a permanent, self-sustaining epidermis.

### Fundamental research benefits

The self-assembly approach can be really useful for fundamental skin studies because this model allows the production of reproducible human skin substitutes, which closely mimic normal or pathological human skin structures and hallmarks. Furthermore, this model brings the possibility to produce many human skin substitutes with only a little piece of skin allowing the reduction of animal models in laboratory. The use of self-assembled skin substitutes, pathological or not, could improve our understanding of cellular complex interactions linked to cutaneous physiology, stem cells interactions, wound healing, skin diseases, dermatopharmacology and angiogenesis. Another particularity of this approach is the possibility to add many cell types according to needs [128-136]. It is also feasible to use pathological cells for the production of pathological skin substitutes [4,13,126,127,137]. The development of such models represents a key component in the fight against these diseases, providing tools that can lead to a better understanding of the pathology and to the development of new pharmaceutical therapies [13].

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