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Endothelialised Tissue Engineered Substitutes Produced by the Self-Assembly Approach for Implantation and Research: Past, Present and Future

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Abstract

Recent innovations in medicine include tissue engineering to produce tissues or organs for replacement/repair surgeries but also to serve as relevant tridimensional research models. For both applications, presence of microvascular networks inside the reconstructed tissues is necessary to obtain structures that are both complex and more complete. Indeed, rapid inosculation of graft to host is essential for positive clinical outcomes but pre-vascularized tissues are also a need to obtain thick tissue where diffusion of nutrients and oxygen cannot be only passive. In the last two decades, the self-assembly approach was developed at LOEX and has allowed breakthroughs in many organ/tissue reconstructions. This unique technique relies on the production of a stroma scaffold by the mesenchymal cells themselves without the need of exogenous materials. Endothelialisation of such tissues shows a great impact not only on graft reperfusion but also in the improvement of research models such as cancer and psoriatic models. The presence of a vascular or lymphatic network now opens the door to the development of complex and configurable models which may be available soon.

Keywords: Tissue engineering; Endothelial cells; Self-assembly

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Introduction

In recent years, the Western world has been confronted with a shortage of adequate tissues for transplants to meet the needs of various pathologies [1]. Indeed, the increase in number of patients requiring repair or replacement of defective organs, mainly related to an ageing demographic and an increasingly sedentary lifestyle of the population, are combined with a decrease in the pool of available organs. This is due to the reasons mentioned above and to the tightening of the selection criteria for tissues suitable for grafting by regulatory agencies. At the request of clinicians, and often with their contribution, scientists have been led to develop innovative technologies to remedy this situation. Among these, tissue engineering seems particularly promising and has undergone significant development in recent decades, even if sometimes chaotic due to the gap between the scientific world and the financial and media worlds who do not think on similar time scales [2-4].

Literature Review

Tissue engineering is primarily based on the use of molecular scaffolding of synthetic or natural origin, onto which cells, potentially from the patient, can be seeded to recreate a functional organ/tissue [5,6]. One of the main pitfalls encountered by tissue engineering has been the ability to graft thick tissues. Indeed, the diffusion limit, which allows a passive exchange of nutrients and oxygen in one direction and of metabolic waste and carbon dioxide in the other, is variable but rarely allows the reconstruction of scaffolds with a thickness of beyond 300-350 µm. Most complex structures, however, require tissues of this thickness and beyond. This is the case when attempting reconstruction of any three-

dimensional organ. Failing to overcome this dilemma would restrict tissue engineering to small, thin tissues and eliminate the possibility of replacing entire organ [7].

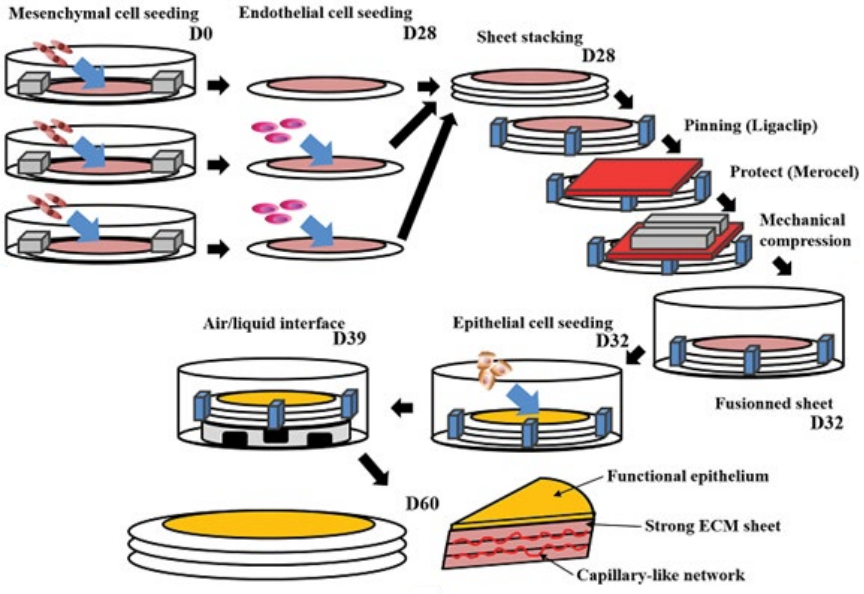
Even if the host's vascular network is capable of invading the reconstructed tissue once grafted, which is not necessarily the case, particularly with synthetic materials; the rate of invasion of the tissue for optimal perfusion of the graft is rarely reached. Partial or total tissue necrosis may be observed. Several solutions have been considered, such as helping the host network to invade the graft more quickly by using proangiogenic growth factors such as Vascular Endothelial Growth Factor (VEGF), or by forming a capillary-like network inside the graft itself before implantation, which can connect to the host by inosculation [8-10].

Discussion

Over the past twenty years, Dr. François A. Auger's group has developed at LOEX a new approach to reconstruct tissues by tissue engineering: the "self-assembly" technique **(Figure 1)** [11]. This technique, which relies on the capacity of stromal cells to produce, secrete and assemble their own extracellular matrix (ECM), makes it possible to build scaffolds very similar to native tissues without the need for exogenous biomaterials which may distance a model from the native tissue. Mechanical properties of transplant tissues are a critical factor for the transplant and for tissue functionality. These properties are fairly satisfactory when using the self-assembly technique [12-15]. In addition, these tissues demonstrated a histological organization and functionality of epithelial cells seeded on stromas, at a level very similar to that observed in native epithelia [12,16]. This is not always the case for epithelial cells when seeded on many biomaterials.

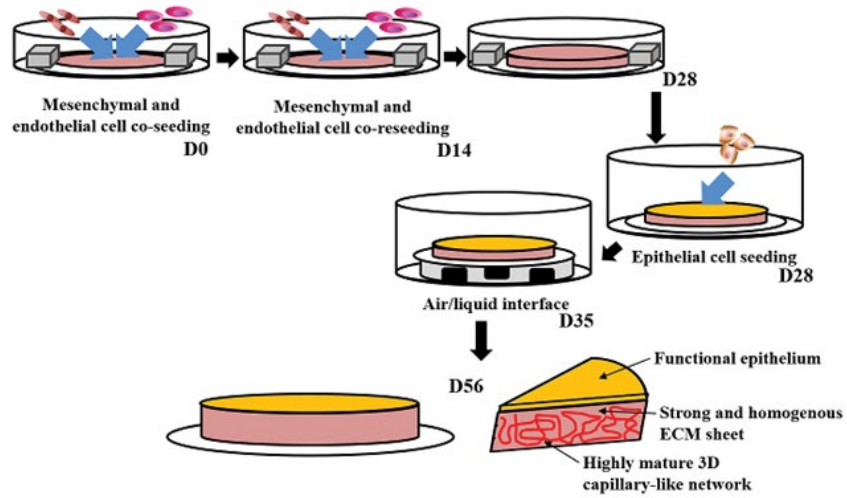
The protocols developed from the self-assembly technique have made it possible to develop a whole range of tissues helpful to compensate for the lack of tissues suitable for grafting. Bilayered skin substitutes consisting of an epidermis made of keratinocytes and a dermis of fibroblasts with their ECM have been used for several years to treat severely burnt patients, but also for those with ulcers or children affected by epidermolysis bullosa [17-19]. Corneal tissues have also been developed to treat patients with blindness associated with corneal clouding following trauma [20]. Blood vessels have been designed simultaneously with skin substitutes, using a similar technique, obtaining a tubular shape by rolling the ECM sheet around a mandrel [21]. Other models are currently under development such as adipose tissue reconstructed from adipose-derived stem/stromal cells (ASC) to serve as filling tissues or biological dressings [22,23]. Bone tissues have also been derived from ASC [24]. More recently, genitourinary tissues, such as the ureters, bladder and urethra have been developed using tubular or flat models, and the vagina as flat models only [12,15,25,26].

In addition to the obvious clinical applications, fundamental study models have been derived from these reconstructed tissues to understand pathologies in a context close to native



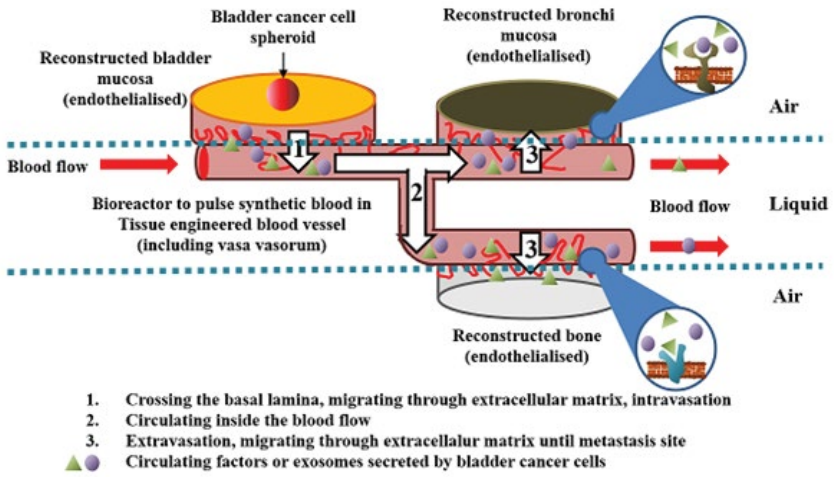
Note: Mesenchymal cells are seeded in three wells of 6-well plates including an anchorage paper device and metal weights. Cells are cultured for 4-6 weeks in the presence of ascorbate to form an ECM sheet. Endothelial cells are then seeded on the top of intermediate and bottom sheets. Sheet is stacked together, pinned with surgical clips and fused under little mechanical load. After 4 days, the fused tissue can be seeded with epithelial cells before one another week of cell culture to allow horizontal proliferation of epithelial cells on the top of the stroma. Tissue is then place at the air/liquid interface for three weeks to achieve epithelial cell stratification and differentiation. This protocol allows the production of tissues with strong ECM without exogenous biomaterials, functional epithelium and a capillary-like network.

Figure 1 Schema of the reconstruction of tissue using the standard protocol of self-assembly.



Note: Mesenchymal and endothelial cells are seeded in one well of 6-well plates including an anchorage paper device and metal weights. Cell is cultured for 2 weeks in the presence of ascorbate before a mesenchymal and endothelial cell reseeding step. Cultures are pursued for 2 additional weeks to form an ECM rich stroma. The tissue can be seeded with epithelial cells before one another week of cell culture to allow horizontal proliferation of epithelial cells on the top of the stroma. Tissue is then place at the air/liquid interface for three weeks to achieve epithelial cell stratification and differentiation. This protocol allows the production of tissues with strong and homogenous ECM without exogenous biomaterials, functional epithelium and a highly mature 3D capillary-like network. For this variant no stacking step is required. Also the cost and complexity of the production are reduced.

Figure 2 Schema of the reconstruction of tissue using the "reseeding" variant protocol of self-assembly.



Note: Branched blood vessels are reconstructed first and placed under flow in bioreactor. The flow is directed from the bladder substitutes to the lung and bone substitutes. The connection from substitutes to reconstructed blood vessels is expected to take 4 days. Bladder cancer spheroids produced by the hanging drop technique are placed on endothelialized reconstructed bladder mucosa, 10 days after the beginning of the air/liquid interface just after the formation of the basal lamina separating the urothelium from the basal lamina. Invasive cancer cells can migrate through the epithelium, cross the basal lamina, invade the stroma and enter in the circulation through micro-vascular network. Cells can then go by the flow until reaching the reconstructed bronchi mucosa or the reconstructed bone. After extravasation, the cancer cell migrates through stroma to create a metastasis. This model allows also the circulation of cancer cell-derived exosomes through the blood flow. These exosomes are known to prepare the ECM to accept the circulating cancer cells.

Figure 3 Schema of a potential model to study cancer metastases spreading from bladder to lungs and bones.

human tissue. Indeed, most studies are performed on inadequate models associated with a low potential for clinical translation [27]. Monolayers of cells in plastic Petri dishes generally do not make it possible to recreate either the complexity of the

organization of the tissue in three dimensions, and therefore, the cell dynamics, polarity in particular, and hence differentiation, or the interactions between cells and the ECM, which a protein coating can very partially represent. Similarly, laboratory animals often show significant differences between species, humans included. The contribution of these models to the advancement of science should not be underestimated; nevertheless, biological complexity obliges us to recognize that it is necessary today to develop more sophisticated models and perhaps in the mid-term, not only to recreate tissues in the laboratory but also to interconnect them, similarly to organ-on-chip technology, which is exciting but too simplistic given the complexity of realistic biological conditions.

In this context, the self-assembly technique has made it possible to study skin pathologies such as hypertrophic scars, systemic sclerosis (scleroderma), melanoma and basal carcinoma, psoriasis, epidermolysis bullosa, neurofibromatosis or skin manifestations of amyotrophic lateral sclerosis (ALS) [19,28-34]. Studies have also been carried out to better understand Fuchs' dystrophy, which affects the corneal endothelium [35]. Models have also been developed to study pathologies of the genitourinary tract, such as bladder cancer, ketamine-induced cystitis, urinary tract infections, as well as infection of an immunocompetent vaginal mucosa model by the human immunodeficiency virus (HIV) [36-38]. These various models could also serve as a solid basis for conducting permeability studies for the pharmaceutical industry as current *in vitro* models are often lacking in complexity.

Whether the objective is to reconstruct tissue for transplantation or to produce models that are as complete as possible, possibly composed of interconnected reconstructed tissues, vascular network formation within the 3D tissue is essential. For example, to study the early stages of metastasis or to measure the dynamics of diffusion of various factors within the tissue [39]. Several approaches have been attempted to recreate a network of capillaries within tissues produced by the self-assembly technique. Initially, human umbilical cord vein endothelial cells (HUVEC) or microvascular endothelial cells (MVEC) were seeded on the surface of ECM sheets which were then stacked to form an endothelialized stroma [40,41]. Although this approach may have given interesting results in the clinic by demonstrating that tissues produced with this approach were reperfused more quickly than non-endothelial tissues, the distribution of capillaries on the surface of the sheets was not optimal. A new technique was therefore introduced **(Figure 2).** The HUVEC/MVEC was seeded along with the stromal cells to produce the sheet and formed a capillary network throughout its entire thickness [42]. It was then possible to reseed endothelial cells and stromal cells on the ECM sheet to thicken it and homogenize the distribution of cells. It was also possible to stack reseeded sheets and have a network of capillaries harmoniously distributed in a relatively thick tissue.

Limits

Even if the tissues produced by the self-assembly method, endothelialized or not, offer real advantages compared to substitutes produced with biomaterials, the fact remains that they have certain drawbacks that it is necessary to mention. Indeed two main problems can arise in the context of the use of tissues reconstructed with the self-assembly method: the first is related to the time required for the production of the tissues and therefore their costs, although significant efforts have been made to decrease these two factors [42,43]. As seen in **Figures 1 and 2**, the time to produce a complete substitute, i.e. with stroma and epithelium, takes about two months. For certain applications which require the production of tissues in an emergency, the technique therefore seems unsuitable even if work is in progress to evaluate the possibility of using decellularized matrices in order to halve the time required [44]. For other applications where time is not a critical factor, such as hypospadias correction surgeries which are performed on an elective basis, this factor is less impactful. The cost issue may, however, put off some surgeons. Nevertheless, if the treatment is evaluated in a long term point of view, the advantages of a bilayered tissue produced with the cells of patients and having a very strong histological and functional similarity can be an important advantage and thus reduce the costs subsequent to the surgery which are often important when using more classic approaches [17]. Another problem arises from the planar or circular configuration of the tissues produced. Some organs require more elaborate morphologies such as the lungs or kidneys, for example, and are difficult to reconstruct using the self-assembly technique. Nevertheless structures as complex as heart valves could be produced with this approach [45,46]. However, no techniques or mix of techniques should be ignored in order to provide the most suitable product to patients, for graftable tissue, or to scientists, for research models.

Future Directions

Nevertheless, many challenges remain to be overcome to recreate thick tissues, that of a thickness beyond the diffusion limit, or to produce an interconnected network of organs/tissues reconstructed by tissue engineering, using the self-assembly technique or others as appropriate. For example, it could be exciting to connect brain organoids with intestinal or bladder tissue due to the numerous pathologies linking the brain and these organs [47,48]. The hypothesis that cancer cells secrete messages that remotely alter the microenvironment of metastasis sites has been developed for many years. It has been revived over the past fifteen years by studies on exosomes [49,50]. Producing tissues from primary cancer sites and studying the diffusion of these exosomes through the bloodstream and their effect on metastatic sites could provide valuable information. It would also be interesting to understand why specific tissues are more inclined to receive circulating cancer cells where others are refractory. The self-assembly technique has also been shown to reconstruct lymphatic vessels, which play a role in these processes [51]. From a technical point of view, such an interconnected tissue network could be built. Several techniques reconstructed many organs/tissues, and blood vessels can be designed and easily interconnected for various calibres **(Figure 3)**.

Conclusion

The medicine of the future has a high potential be a personalized medicine where each person can receive appropriate treatment for the pathology that affects them, adapted to each unique profile [52-55]. Contrary to what some people imagine, such a development would reduce health costs by avoiding the use of expensive drugs that may not prove useful for a specific person and therefore by shortening treatment times and reducing side effects. Unlike the models currently used, the development of complex research models where all aspects could be parameterized and controlled, unlike animal models, and this from a patient's own cells, will allow these feats that were still unimaginable a half-century ago but are today within our reach. Also, people will be able to have access to a range of tissues reconstructed in the laboratory with their own cells and that are therefore perfectly immune-compatible, having a degree of differentiation, and therefore functionality, as high as possible, allowing a return to a better quality of life. The development of tissue engineering for direct clinical applications and for the advancement of knowledge has the potential to change the face of medicine. It could be comparable to the discoveries of antibiotics and vaccines. Sky is the limit.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Considerations

Ethical review and approval were waived for this study, due to no human subject or animal was used.

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References

1 Lewis A, Koukoura A, Tsianos GI, Gargavanis AA, Nielsen AA, et al. (2021) Organ donation in the US and Europe: The supply vs demand imbalance. Transplant Rev 35: 100-585.

2 Langer R, Vacanti JP (1993) Tissue engineering. Science 260: 920-926.

3 Langer R, Vacanti J (2016) Advances in tissue engineering. J Pediatr Surg 51: 8-12.

4 O'Donnell BT, Ives CJ, Mohiuddin OA, Bunnell BA (2019) Beyond the present constraints that prevent a wide spread of tissue engineering and regenerative medicine approaches. Front Bioeng Biotechnol 7: 95.

5 Lynch CR, Kondiah PPD, Choonara YE (2021) Advanced strategies for tissue engineering in regenerative medicine: A biofabrication and biopolymer perspective. Molecules 26: 2518.

6 Caneparo C, Chabaud S, Bolduc S (2021) Reconstruction of vascular and urologic tubular grafts by tissue engineering. Processes 9: 513.

7 Sekine H, Okano T (2020) Capillary networks for bio-artificial three-dimensional tissues fabricated using cell sheet based tissue engineering. Int J Mol Sci 22: 92.

8 Auger FA, Gibot L, Lacroix D (2013) The pivotal role of vascularization in tissue engineering. Annu Rev Biomed Eng 15: 177-200.

9 Masson-Meyers DS, Tayebi L (2021) Vascularization strategies in tissue engineering approaches for soft tissue repair. J Tissue Eng Regen Med. Ahead of print.

10 Wang B, Patnaik SS, Brazile B, Butler JR, Claude A, et al. (2015) Establishing early functional perfusion and structure in tissue engineered cardiac constructs. Crit Rev Biomed Eng 43: 455-471.

11 Saba I, Jakubowska W, Bolduc S, Chabaud S (2018) Engineering tissues without the use of a synthetic scaffold: A twenty-year history of the self-assembly method. Biomed Res Int 2018: 1-13.

12 Bouhout S, Perron E, Gauvin R, Bernard G, Ouellet G, et al. (2010) *In vitro* reconstruction of an autologous, watertight, and resistant vesical equivalent. Tissue Eng Part A 16: 1539-1548.

13 Gauvin R, Guillemette M, Galbraith T, Bourget JM, Larouche D, et al. (2011) Mechanical properties of tissue-engineered vascular constructs produced using arterial or venous cells. Tissue Eng Part A 17:2049-2059.

14 Tondreau MY, Laterreur V, Gauvin R, Vallières K, Bourget JM, et al. (2015) Mechanical properties of endothelialized fibroblast-derived vascular scaffolds stimulated in a bioreactor. Acta Biomater 18: 176-185.

15 Jakubowska W, Chabaud S, Saba I, Galbraith T, Berthod F, et al. (2020) Prevascularized tissue-engineered human vaginal mucosa: *In vitro* optimization and *in vivo* validation. Tissue Eng Part A 26: 811-822.

16 Auger FA, Berthod F, Moulin V, Pouliot R, Germain L (2004) Tissue-engineered skin substitutes: From *in vitro* constructs to *in vivo* applications. Biotechnol Appl Biochem 39: 263-275.

17 Germain L, Larouche D, Nedelec B, Perreault I, Duranceau L, et al. (2018) Autologous bilayered self-assembled skin substitutes (SASSs) as permanent grafts: A case series of 14 severely burned patients indicating clinical effectiveness. Eur Cell Mater 36: 128-141.

18 Boa O, Cloutier CB, Genest H, Labbé R, Rodrigue B, et al. (2013) Prospective study on the treatment of lower-extremity chronic venous and mixed ulcers using tissue-engineered skin substitute made by the self-assembly approach. Adv Skin Wound Care 26: 400-409.

19 Dakiw PA, Larouche D, Ghani K, Bisson F, Cortez GS, et al. (2018) Translating the combination of gene therapy and tissue engineering for treating recessive dystrophic epidermolysis bullosa. Eur Cell Mater 35: 73-86.

20 Proulx S, d'Arc Uwamaliya J, Carrier P, Deschambeault A, Audet C, et al. (2010) Reconstruction of a human cornea by the self-assembly approach of tissue engineering using the three native cell types. Mol Vis 2010 16: 2192-2201.

21 L'Heureux N, Pâquet S, Labbé R, Germain L, Auger FA (1998) A completely biological tissue-engineered human blood vessel. FASEB J 12: 47-56.

22 Proulx M, Aubin K, Lagueux J, Audet P, Auger M, et al. (2015) Magnetic resonance imaging of human tissue-engineered adipose substitutes. Tissue Eng Part C Methods 21: 693-704.

23 Morissette MP, Maux A, Laterreur V, Mayrand D, Gagné LV, et al. (2015) Enhancing repair of full-thickness excisional wounds in a murine model: Impact of tissue-engineered biological dressings featuring human differentiated adipocytes. Acta Biomater 22: 39-49.

24 Kawecki F, Galbraith T, Clafshenkel WP, Fortin M, Auger FA, et al. (2021) *In vitro* prevascularization of self-assembled human bone-like tissues and preclinical assessment using a rat calvarial bone defect model. Materials 14: 2023.

25 Cattan V, Bernard G, Rousseau A, Bouhout S, Chabaud S, et al. (2011) Mechanical stimuli-induced urothelial differentiation in a human tissue-engineered tubular genitourinary graft. Eur Urol 60: 1291-1298.

26 Orabi H, Saba I, Rousseau A, Bolduc S (2017) Novel three-dimensional autologous tissue-engineered vaginal tissues using the self-assembly technique. Transl Res 180: 22-36.

27 Bédard P, Gauvin S, Ferland K, Caneparo C, Pellerin È, et al. (2020) Innovative human three-dimensional tissue-engineered models as an alternative to animal testing. Bioengineering 7: 115.

28 Simon F, Bergeron D, Larochelle S, Lopez-Vallé CA, Genest H, et al. (2012) Enhanced secretion of TIMP-1 by human hypertrophic scar keratinocytes could contribute to fibrosis. Burns 38: 421-427.

29 Corriveau MP, Boufaied I, Lessard J, Chabaud S, Senécal JL, et al. (2009) The fibrotic phenotype of systemic sclerosis fibroblasts varies with disease duration and severity of skin involvement: Reconstitution of skin fibrosis development using a tissue engineering approach. J Pathol 217: 534-542.

30 Gibot L, Galbraith T, Huot J, Auger FA (2013) Development of a tridimensional microvascularized human skin substitute to study melanoma biology. Clin Exp Metastasis 30: 83-90.

31 Bourland J, Fradette J, Auger FA (2018) Tissue-engineered 3D melanoma model with blood and lymphatic capillaries for drug development. Sci Rep 8: 13191.

32 Jean J, Lapointe M, Soucy J, Pouliot R (2008) Development of an in vitro psoriatic skin model by tissue engineering. J Dermatol Sci 53: 19-25.

33 Roy V, Lamontagne R, Talagas M, Touzel-Deschênes L, Khuong HT, et al. (2021) Biofabrication of a three dimensional human-based personalized neurofibroma model. Biotechnol J 10: e2000250.

34 Paré B, Touzel-Deschênes L, Lamontagne R, Lamarre MS, Scott FD, et al. (2015) Early detection of structural abnormalities and cytoplasmic accumulation of TDP-43 in tissue-engineered skins derived from ALS patients. Acta Neuropathol Commun 3: 5.

35 Goyer B, Thériault M, Gendron SP, Brunette I, Rochette PJ, et al. (2018) Extracellular matrix and integrin expression profiles in fuchs endothelial corneal dystrophy cells and tissue model. Tissue Eng Part A 24: 607-615.

36 Ringuette GC, Bernard G, Chabaud S, Couture A, Langlois A, et al. (2017) Tissue-engineered human 3D model of bladder cancer for invasion study and drug discovery. Biomaterials 145: 233-241.

37 Bureau M, Pelletier J, Rousseau A, Bernard G, Chabaud S, et al. (2015) Demonstration of the direct impact of ketamine on urothelium using a tissue engineered bladder model. Can Urol Assoc J 9: E613-617.

38 Saba I, Barat C, Chabaud S, Reyjon N, Leclerc M, et al. (2021) Immunocompetent Human 3D organ-specific hormone-responding vaginal mucosa model of HIV-1 infection. Tissue Eng Part C Methods 27: 152-166.

39 Neophytou CM, Panagi M, Stylianopoulos T, Papageorgis P (2021) The role of tumor microenvironment in cancer metastasis: Molecular mechanisms and therapeutic opportunities. Cancers 13: 2053.

40 Guillemette MD, Gauvin R, Perron C, Labbé R, Germain L, et al. (2010) Tissue-engineered vascular adventitia with vasa vasorum improves graft integration and vascularization through inosculation. Tissue Eng Part A 16: 2617-2626.

41 Imbeault A, Bernard G, Rousseau A, Morissette A, Chabaud S, et al. (2013) An endothelialized urothelial cell-seeded tubular graft for urethral replacement. Can Urol Assoc J 7: E4-E9.

42 Chabaud S, Rousseau A, Marcoux TL, Bolduc S (2017) Inexpensive production of near-native engineered stromas. J Tissue Eng Regen Med 11: 1377-1389.

43 Chabaud S, Marcoux TL, Deschênes-Rompré MP, Rousseau A, Morissette A, et al. (2015) Lysophosphatidic acid enhances collagen deposition and matrix thickening in engineered tissue. J Tissue Eng Regen Med 9: E65-75.

44 Beaudoin CC, Goyer B, Perron C, Guignard R, Larouche D, et al. (2017) *In vivo* evaluation and imaging of a bilayered self-assembled skin substitute using a decellularized dermal matrix grafted on mice. Tissue Eng Part A 23: 313-322.

45 Tremblay C, Ruel J, Bourget JM, Laterreur V, Vallières K, et al. (2014) A new construction technique for tissue-engineered heart valves using the self-assembly method. Tissue Eng Part C Methods 20: 905-915.

46 Picard-DM, Ruel J, Galbraith T, Tremblay C, Kawecki F, et al. (2017) Tissue-engineered tubular heart valves combining a novel precontraction phase with the self-assembly method. Ann Biomed Eng 45: 427-438.

47 Tish MM, Geerling JC (2020) The Brain and the bladder: Forebrain control of urinary (in) continence. Front Physiol 11: 658.

48 Jörg E, Sartori AM, Hofer AS, Baumann CR, Kessler TM (2020) Deep brain stimulation effects on lower urinary tract function: Systematic review and meta-analysis. Parkinsonism Relat Disord 79: 65-72.

49 Liu Y, Shi K, Chen Y, Wu X, Chen Z, et al. (2021) Exosomes and their role in cancer progression. Front Oncol 11: 639-1159.

50 Sung BH, Parent CA, Weaver AM (2021) Extracellular vesicles: Critical players during cell migration. Dev Cell 2: S1534-1580.

51 Gibot L, Galbraith T, Bourland J, Rogic A, Skobe M, et al. (2017) Tissue-engineered 3D human lymphatic microvascular network for *in vitro* studies of lymphangiogenesis. Nat Protoc 12: 1077-1088.

52 Bloss CS, Jeste DV, Schork NJ (2011) Genomics for disease treatment and prevention. Psychiatr Clin North Am 34: 147-166.

53 Kamran SC, Efstathiou JA (2021) Current state of personalized genitourinary cancer radiotherapy in the era of precision medicine. Front Oncol 11: 675-1311.

54 Ma YS, Yang XL, Xin R, Wu TM, Shi Y, et al. (2021) The power and the promise of organoid models for cancer precision medicine with next-generation functional diagnostics and pharmaceutical exploitation. Transl Oncol 14: 101-126.

55 Wang Y, Zheng D (2021) The importance of precision medicine in modern molecular oncology. Clin Genet 99: 13998.