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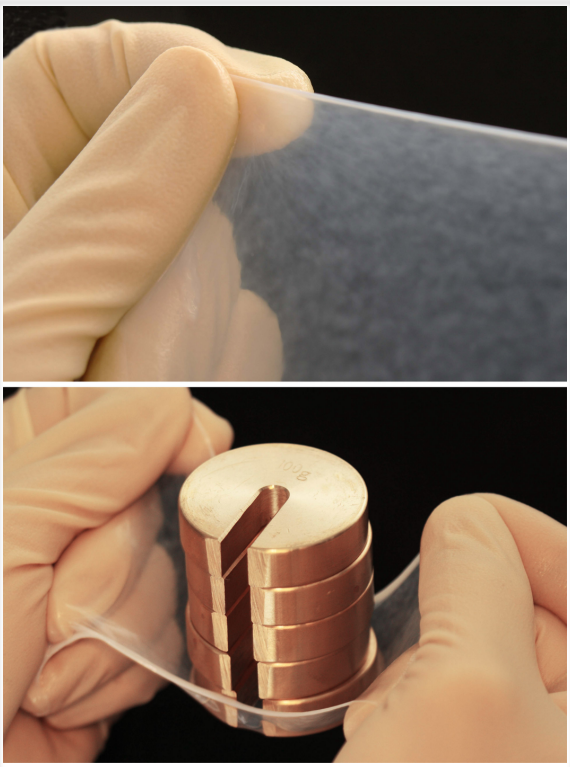
Keywords/expertise:

- Human cell culture
- Bioreactors
- Cardiovascular biology
- Endothelium
- Fibroblasts
- Smooth muscle cells
- Tissue-engineering
- Bioengineering
- Regenerative Medicine
- Cell-based therapies
- Vascular graft
- Extracellular matrix
- Collagen
- Histology
- Immunofluorescence
- Electron microscopy
- Mechanical properties
- Mechanical stimulation

Research Interests:



A sheet of human CAM is a true bio-material that can have remarkable mechanical properties while avoiding immune and inflammatory reactions. (from review[8])

Cell-assembled extracellular matrix (CAM)

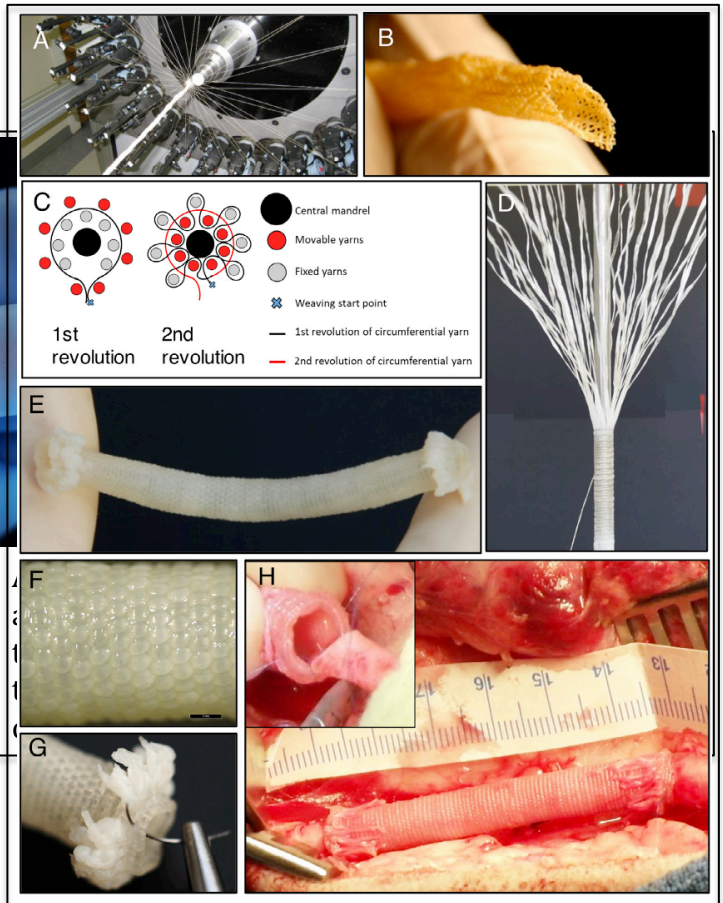
My interests have always been centered on the use of human cells to produce tissue-engineered constructs for therapeutic applications. [1, 2] My experience has been focused on the field of cardiovascular tissue engineering but I like to also dabble in other areas. For 30 years, I have worked on using the extracellular matrix produced by normal human cells in culture. This Cell-Assembled Matrix (CAM) is a unique material that can have remarkable mechanical strength when produced in the right culture conditions. I have used this CAM to produce small-diameter, tissue-engineered vascular grafts (TEVG) that display burst pressures similar that of native human blood vessels, but without the need for any exogenous scaffolding such a synthetic polymers or animal/cadaveric tissues. [2-4] Because they do not include foreign materials, because the CAM is not chemically or otherwise denatured, and because the CAM is of human origin, we would not expect constructs build following this approach to trigger a response from neither the adaptive nor the innate immune system. I spent 15 years as the co-founder and Chief Scientific Officer of Cytograft Tissue Engineering (San Francisco, California) driving the R&D effort to bring these TEVGs to the clinic. These autologous CAM-based TEVGs were the first completely biological TEVGs to be implanted in humans and the first TEVGs to be implanted in the high-pressure arterial system. [6, 7] These TEVGs have shown remarkable patency as arteriovenous shunt for hemodialysis of end stage renal disease patients with durability of up to 3 years. [9] Allogeneic and non-living TEVGs have also been successfully used in patients. [10, 11]

This last development suggest that allogeneic tissues can be used as the basic strategy to develop CAM-based therapies. This approach is much more commercially feasible than an autologous approach, which, while possibly better, has become economically unrealistic considering today's regulatory requirement. Consequently, our approach also includes using "devitalized" tissues (non-living). In a recent paper, we have shown that devitalization by freeze/thaw and air-drying/rehydration appears to preserve the quality of the extracellular matrix. [12] Another advantage of working from a bank of donor cells is to avoid inter-individual variability in the ability of cells to produce ECM *in vitro*. One of our recent papers has shown the variability in strength and composition of the CAM in GMP conditions produced by 21 patients. [13][Magnan, 2018 #8273] While these differences can be managed with the right quality control strategy, using a more controlled cell line is certainly an advantage.

A CAM sheet can also be used to build heat valves (project driven by Dr. Kawecki). It can also be used as a "biopaper" to print cells on it and assemble then in more complex structures by stacking. [14] The

CAM could be wrapped around or otherwise used to mask a synthetic implant to control its interaction with the immune system and or the blood of the patient (2 projects: one driven by Dr. Schlund, one in collaboration with an artificial heat company).

Thread-Based Tissue Engineering: or how to make Human Textiles.



The CAM is produced at the bottom of culture flasks as sheets of various shapes and sizes. The sheets can be cut, stacked, folded or, like in the case of the above-mentioned blood vessel, rolled to create a variety of tissues. In the specific case of TEVGs, the sheet-based approach has a significant drawback: the need for a long culture period (maturation) to allow the cells to fuse the different layers of sheets together. In addition, fusion is limited to a certain depth because of transport issues, which entails a multi-step maturation strategy to create thicker tissues. At BioTis, I have been developing a new production method based on the use of threads of CAM instead of sheets. [5] Taking advantage of various textile technologies (weaving, knitting, braiding), we can literally produce **human textiles** with a wide range of geometries, porosities, (anisotropic) mechanical properties, and all that with regional tunability. In addition, because threads can be connected to each other, the size of the constructs is no longer limited to the size of culture flasks. But the most interesting advantage of this thread-based approach is the fact that the cohesion of the constructs does not rely on a maturation phase and is not hindered by transport limitations. Indeed, the textiles are ready for use as soon as they are assembled, which takes the production time of the TEVG from over 6 months to about 2 months (or even less). The development of a thread-based TEVG is one of the main research projects in my group. We have shown that these human threads have long lives *in vivo*. This was shown in nude rats that still have a functional innate immune response and can degrade denatured collagen. [15] In order to work in large animal models, we have developed ovine CAM (from ovine fibroblasts). [16] We have built ovine TEVG and are in the process of testing them as end-to-end as well as arterio-venous shunts in an allogeneic context in the sheep.

We have used a Thread-Based Tissue Engineering approach using a different sheet of native ECM: the human amniotic membrane (HAM). This material can be obtained directly in maternity wards and, without chemical denaturation, can be processed into sheets and threads. [17] This material is obvious less expensive than the CAM but has its own limitations (variability and accessibility). Nonetheless, we have shown that HAM-based textiles can be produce and woven TEVG from this material have the mechanical properties necessary to *in vivo* testing. [18]

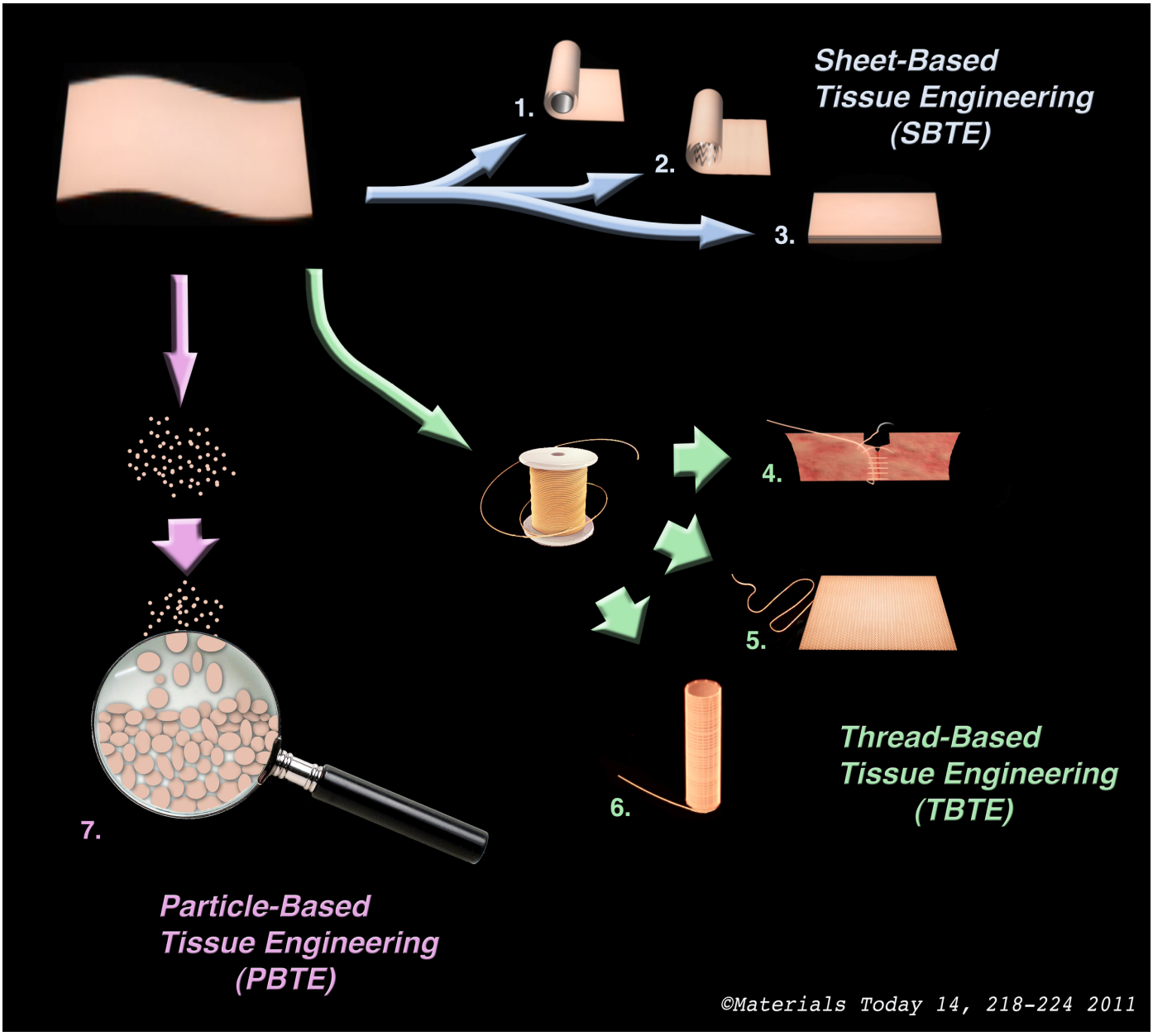
Because the CAM can provide near-native conjunctive tissue in a wide range of configurations[19], we are also exploring other applications in Regenerative Medicine such as creating a support tissue to repair pelvic organ prolapse, producing an esophageal implant, or making surgical sutures.

Particle-Based Tissue Engineering: moldable and injectable.

Human CAM can also be produced in the form of particles that can be molded or injected. This particle-based approach can be used to produce porous structures for tissue engineering or can even be injected directly *in vivo* to create new tissues. This has obvious aesthetic applications but also tissue reconstruction and cell-delivery applications.

Below is schematic representation of the various building strategies we have developed using CAM:

Completely biological, human, textile-based, tissue-engineered vascular grafts. A, B) Automated manufacturing of a braided tube with human threads (48 spools). While this demonstrated the feasibility of automation, the resulting tube was clearly too porous for use as a vascular graft. C) Schematic representation of the basic weaving technique used with a manual circular loom. One circumferential yarn (weft) was inserted between a movable and a fixed set of tensioned yarns (warp) to create the woven tube. D) Production of the TEVG with, partly rehydrated, devitalized yarns: 49 longitudinal ribbons and one double-filament thread (2 ribbons twisted together at 5 $rev \cdot cm^{-1}$) as circumferential thread. E) Vascular grafts were fully rehydrated before removal from the mandrel yielding a robust and flexible tube. F) The tightly packed yarn produced a dense, homogenous, and watertight wall. G) The ends of the graft were "finished" to produce a clear and even ring for suturing (internal diameter of 4.2 mm). H) A human woven TEVG (internal diameter 4.2 mm) was implanted in the carotid of a sheep as interpositional using 6-0 Prolene® using standard surgical techniques (inset). The graft was leak-proof and allowed normal blood flow. (Figure 6 of [5])



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- Mechanical properties
- Mechanical stimulation
- Pre-clinical studies
- Clinical trials
- Translational medicine
- Entrepreneurship
- Startup
- Patents
- Technology transfer

Cited publications:

[1] L'Heureux, N., Germain, L., Labbe, R., Auger, F.A., *In vitro* construction of a human blood vessel from cultured vascular cells: a morphologic study, *Journal of Vascular Surgery* 17(3) 499-509 (1993).
[2] L'Heureux, N., Paquet, S., Labbe, R., Germain, L., Auger, F.A., A completely biological tissue-engineered human blood vessel, *FASEB J.* 12(1) 47-56 (1998).
[3] L'Heureux, N., Dusserre, N., Konig, G., Victor, B., Keire, P., Wight, T.N., Chronos, N.A., Kyles, A.E., Gregory, C.R., Hoyt, G., Robbins, R.C., McAllister, T.N., Human tissue-engineered blood vessels for adult arterial revascularization, *Nat. Med.* 12(3) 361-5 (2006).
[4] Konig, G., McAllister, T.N., Dusserre, N., Garrido, S.A., Iyican, C., Marini, A., Fiorillo, A., Avila, H., Wystrychowski, W., Zagalski, K., Maruszewski, M., Jones, A.L., Cierpka, L., de la Fuente, L.M., L'Heureux, N., Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery, *Biomaterials* 30(8) 1542-50 (2009).
[5] Magnan, L., Labrunie, G., Fenelon, M., Dusserre, N., Foulc, M.P., Lafourcade, M., Svahn, I., Gontier, E., Velez, V.J., McAllister, T.N., L'Heureux, N., Human textiles: A cell-synthesized yarn as a truly "bio" material for tissue engineering applications, *Acta Biomater.* 105 111-120 (2020).
[6] L'Heureux, N., McAllister, T.N., de la Fuente, L.M., Tissue-engineered blood vessel for adult arterial revascularization, *N. Eng. J. Med.* 357(14) 1451-3 (2007).
[7] McAllister, T.N., Maruszewski, M., Garrido, S.A., Wystrychowski, W., Dusserre, N., Marini, A., Zagalski, K., Fiorillo, A., Avila, H., Manglano, X., Antonelli, J., Kocher, A., Zembala, M., Cierpka, L., de la Fuente, L.M., L'Heureux, N., Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study, *Lancet* 373(9673) 1440-6 (2009).
[8] Peck, M., Gebhart, D., Dusserre, N., McAllister, T.N., L'Heureux, N., The evolution of vascular tissue engineering and current state of the art, *Cells Tissues Organs* 195(1-2) 144-58 (2012).
[9] (!!! INVALID CITATION !!! 8).
[10] Wystrychowski, W., Cierpka, L., Zagalski, K., Garrido, S., Dusserre, N., Radochonski, S., McAllister, T.N., L'Heureux, N., Case study: first implantation of a frozen, devitalized tissue-engineered vascular graft for urgent hemodialysis access, *J Vasc Access* 12(1) 67-70 (2011).
[11] Wystrychowski, W., McAllister, T.N., Zagalski, K., Dusserre, N., Cierpka, L., L'Heureux, N., First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access, *J. Vasc. Surg.* 60(5) 1353-1357 (2014).
[12] Magnan, L., Labrunie, G., Marais, S., Rey, S., Dusserre, N., Bonneu, M., Lacomme, S., Gontier, E., L'Heureux, N., Characterization of a Cell-Assembled extracellular Matrix and the effect of the devitalization process, *Acta Biomater.* 82 56-67 (2018).
[13] Kawecki, F., Gluais, M., Claverol, S., Dusserre, N., McAllister, T.N., L'Heureux, N., Inter-Donor Variability of Extracellular Matrix Production in Long-Term Cultures of Human Fibroblasts, *Biomaterials Science* (2022).
[14] Oliveira, H., Médina, C., Labrunie, G., Dusserre, N., Catros, S., Magnan, L., Handschin, C., Stachowicz, M.L., Fricain, J.C., L'Heureux, N., Cell-assembled extracellular matrix (CAM): a human biopaper for the biofabrication of pre-vascularized tissues able to connect to the host circulationin vivo, *Biofabrication* 14(1) (2021).
[15] Magnan, L., Kawecki, F., Labrunie, G., Gluais, M., Izotte, J., Marais, S., Foulc, M.P., Lafourcade, M., L'Heureux, N., In vivo remodeling of human cell-assembled extracellular matrix yarns, *Biomaterials* 273 120815 (2021).
[16] Torres, Y., Gluais, M., Da Silva, N., Rey, S., Grémare, A., Magnan, L., Kawecki, F., L'Heureux, N., Cell-assembled extracellular matrix (CAM) sheet production: Translation from using human to large animal cells, *J Tissue Eng* 12 2041731420978327 (2021).
[17] Gremare, A., Jean-Gilles, S., Musqui, P., Magnan, L., Torres, Y., Fenelon, M., Brun, S., Fricain, J.C., L'Heureux, N., Cartography of the mechanical properties of the human amniotic membrane, *J Mech Behav Biomed Mater* 99 18-26 (2019).
[18] Grémare, A., Thibes, L., Gluais, M., Torres, Y., Potart, D., Da Silva, N., Dusserre, N., Fénelon, M., Sentilhes, L., Lacomme, S., Svahn, I., Gontier, E., Fricain, J.C., L'Heureux, N., Development of a vascular substitute produced by weaving yarn made from human amniotic membrane, *Biofabrication* 14(4) (2022).
[19] Peck, M., Dusserre, N., McAllister, T.N., L'Heureux, N., Tissue engineering by self-assembly, *Materials Today* 14(5) 218-224 (2011).

Patents:

1- McAllister, T., and L'Heureux, N. Tissue engineered cellular sheets, and methods of making same. USPTO Patent No. 8,076,137 (December 13, 2011).
2- McAllister, T., and L'Heureux, N. Bioreactor for the manufacture of tissue engineered blood vessels. USPTO Patent No. 7,744,526 (June 29, 2010).
3- McAllister, T., and L'Heureux, N. Tissue engineered cellular sheets, methods of making and use thereof. USPTO Patent No. 7,504,258 (March 17, 2009).
4- McAllister, T., and L'Heureux, N. Method of culturing cells to produce a tissue sheet. USPTO Patent No. 7,166,464 (January 23, 2007).
5- McAllister, T., and L'Heureux, N. Tissue engineered blood vessels and apparatus for their manufacture. USPTO Patent No. 7,112,218 (September 26, 2006).
6- McAllister, T., and L'Heureux, N. Tissue engineered blood vessels and methods and apparatus for their manufacture. USPTO Patent No. 6,503,273 (January 7, 2003).
7- L'Heureux, N., Auger, F.A., and Germain, L. Production of a contractile smooth muscle. USPTO Patent No. 5,618,718 (April 8, 1997).

Education:

1991-96	Ph.D. in Molecular & Cell Biology	Université Laval, Québec, Canada Laboratoire d'Angiogénèse Expérimentale / LOEX
1994	Internship (3 months)	Université Louis Pasteur de Strasbourg, France. UMR CNRS 7034 (Pr. J-C Stoclet)
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BIOMAT : The French association for the development of biomaterials, Tissue Engineering and Regenerative Medicine: http://www.biomat.fr

ISACB (Board Member): International Society for Applied Cardiovascular Biology: http://isacb.org