and envisioning a radical change in the management of traumatic amputations and upper extremity transplantation.

Vascularized tongue extracellular matrix production in a porcine model

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Background: Tongue reconstruction is a true challenge due to its very specific and complex functions, motor and sensory. Despite the most advanced autologous surgical techniques, functional outcomes remain poor. To overcome the current limitations, vascularized composite tissue engineering is thus a promising solution.

Methods: Seven porcine tongues were harvested with their vascular lingual arteries and nervous pedicles. Perfusion decellularization was achieved with a detergent-based protocol. Cell removal was established by conventional stainings and DNA quantification. The extracellular matrix structural preservation was appreciated with Masson's trichrome and laminin stainings, three-dimensional histology, and scanning electron microscopy. Vascular bed preservation and access was assessed by angioscopy and Indian ink gel injection. To study cell compatibility, tongue extracellular matrix biopsies were cultured with C2C12 muscle progenitor cells. In vivo biocompatibility was examined by scaffold implantation in rat quadriceps for 4 weeks.

Results: Complete decellularization of porcine tongues could be obtained within 8 days. Cell clearance was confirmed by absence of nuclei on hemotoxylin and eosin sections, and a significant DNA content reduction. Masson's trichrome staining, three-dimensional histology, and scanning electron microscopy showed a well-preserved microscopic structure. Laminin, an important factor for muscle regeneration, was highly preserved. The vascular bed was patent, for both large and small vessels. Seeded cells were viable and implanted scaffolds demonstrated a satisfying integration in the recipient muscle, at macro- and microscopic levels.

Conclusion: We could produce complete porcine tongue graft extracellular matrix, with a patent and accessible vascular tree, which was cyto- and bio-compatible. These results enable future research in whole-tongue vascularized composite tissue engineering.

Vascularized human finger decellularization: a subunit approach to hand tissue engineering

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Background: In hand surgery, conventional autologous techniques are limited for the reconstruction of lost digits, with a high impact on hand function. Finger subunit allotransplantation has been described, but is restricted by limited applications due to immunosuppressive treatment. From previous report in acellular porcine ear graft matrix production, we applied the perfusion-decellularization technique to human finger subunit grafts obtained from cadaveric source, then extended to whole hands.

Methods: Nine long-digit grafts were procured postmortem, along with their collateral ulnar and radial pedicles, from four fresh human donors at the Department of Anatomy. The specimens were decellularized by sequential arterial perfusions of heparinized saline, 1% sodium dodecyl sulfate, isopropanol, phosphate-buffered saline, and type-I DNAse. Cellular clearance was assessed by DNA quantification and hematoxylin and eosin staining. Extracellular matrix preservation was assessed by Masson's trichrome for soft tissues and bone mineral density for phalangeal bones. Acellular samples from skin, bone, and tendon were cultured with NIH-3 T3 fibroblastic cell line for 4 days, and examined with live/dead staining. Finally, a scaffold was re-anastomosed in a porcine recipient, for a short reperfusion study. Finally, decellularization was applied to three whole human hands, and vascular tree assessed by angio-computed tomographic scan.

Results: Digital grafts were successfully decellularized, with quick epidermolysis, nail loss, and complete bleaching. Cell clearance was demonstrated by absence of nuclei on hematoxylin and eosin sections, and an overall 95.3 % DNA content reduction, compared to native tissues ($p < 0.0001$). Masson's trichrome staining showed microscopic structural preservation. Decellularized phalanx mean bone mineral density was 510 mg hydroxyapatite/cm^3, similar to control. Seeded NIH-3 T3 cells were viable and homogeneously distributed on all scaffolds. In vivo, blood quickly reperfused the whole scaffold; after 3 h, the vascular tree was still patent, as demonstrated by fluoroscopy, fingertip pulse, and oxygen saturation at 93%. The extension to a whole-hand graft decellularization was achieved successfully, with an excellent preservation of the superficial and deep vascular tree.

Conclusion: Following our subunit approach strategy, we could produce finger and hand extracellular matrix scaffolds

from human cadaveric source, with a preserved and perfusable vascular tree. These results could represent a true alternative to upper extremity allotransplantation.

Extended normothermic ex-situ limb perfusion preserves limbs viable and functional up to 44 h

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Background: Ischemia and reperfusion injury remains one of the major limiting factors for the success of both replantation and vascularized composite allotransplantation. Compared to cold storage of preserved/procured organs, normothermic ex-situ perfusion is a novel approach that prolongs viability of the limb by maintaining physiologic cellular metabolism avoiding the deleterious effects of both hypoxia and cooling. This study aims to develop an ex-situ normothermic limb perfusion protocol to preserve the viability and function of amputated limbs for over 24 h.

Methods: A total of 23 swine limbs were perfused using an oxygenated colloid solution at 38°C containing washed red blood cells. The first 13 limbs were used to optimize the perfusion protocol. The subsequent five limbs (Group A) were perfused for 12 h and the following five (Group B) as long as muscle contractility/peripheral perfusion was present. Electrolytes were balanced by partial perfusate exchanges. Limb viability was compared in the two groups by muscle contractility, compartment pressure, tissue oxygen saturation, indocyanine green angiography, and thermography.

Results: Perfused limbs were able to retain physiological parameters and function for 12 h in Group A and up to 44 (24–44) h in Group B. Limbs in Group A had lower final weight increase ($0.54\% \pm 0.07$ vs $14.11\% \pm 16.27$; $p=0.008$) and compartment pressure compared to Group B (16.23 ± 7.10 vs 24.75 ± 7.79; $p=0.175$). Final myoglobin and creatine kinase mean values were lower in Group A compared to Group B (875 ± 291.4 ng/mL vs 1010.6 ± 323.6 ng/mL and $53{,}344 \pm 14850.34$ U/L vs $71{,}881 \pm 20{,}475$ ng/mL). In Group B, thermography and tissue oxygen saturation were significantly higher than in Group A (respectively, $35.37°C \pm 0.69°C$ vs $33°C \pm 1.44°C$; $p = 0.01$ and $69.31\% \pm 9.3\%$ vs $58.69\% \pm 8.4\%$; $p=0.048$). Contractility and indocyanine green angiography were comparable in the two groups.

Conclusion: The normothermic perfusion protocol has the potential to significantly impact outcomes in limb replantation and transplantation. Our results suggest that limbs in the 12-h perfusion group retained better physiologic parameters at the end-point. However, the continuous optimization of the protocol allowed to maintain limb's function and preserved peripheral perfusion for up to 44 h.

Markov decision analytic model of the cost-utility of composite tissue allotransplantation, myoelectric prosthetics, and body-powered prosthetics in bilateral transradial amputation

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Background: Canadians' bilateral upper limb amputees have access to body-powered prosthetics, which limit functional improvement and are often rejected, and two emerging technologies: myoelectric prosthetics and composite tissue allotransplantation. The purpose of this study is to evaluate the cost-effectiveness of these three strategies from the Canadian Health System perspective.

Methods: A state transition cohort decision analytic model was used to evaluate the treatment options for improving functional status in bilateral upper limb amputees. The model reflected three treatment strategies: (1) bilateral body-powered prosthetics, (2) bilateral myoelectric prosthetics, and (3) bilateral composite tissue allotransplantation. Both quality-adjusted life years and costs were modeled over a lifetime time horizon. Utilities, probabilities, and costs were identified through a targeted literature search, local case costing, or expert opinion. Model validity was tested, base case incremental cost-effectiveness ratios were calculated, and one- and two-way and probabilistic sensitivity analyses were conducted.

Results: Over a 44-year time horizon, the preferred strategy was body-powered prosthetics, which resulted in 14.45 quality-adjusted life years at a cost of CAD281,795. Relative to body-powered prosthetics, myoelectric prosthetics and composite tissue allotransplantation had incremental cost-effectiveness ratios above the standard willingness-to-pay threshold (CAD50,000 per quality-adjusted life years) at CAD75,895 and CAD780,061 per quality-adjusted life years, respectively. Composite tissue allotransplantation was neither favored nor cost-effective in one-way sensitivity analysis. The model was sensitive to the costs and utilities of myoelectric prosthetics and body-powered prosthetics on both one- and two-way analyses. When myoelectric prosthetics device cost is $<$CAD31,000, myoelectric prosthetics is the preferred strategy over body-powered prosthetics and when $<$CAD25,000, body-powered prosthetics is not cost-effective and removed from the model. PSA demonstrated that body-powered prosthetics and myoelectric prosthetics met the willingness-to-pay threshold (CAD50,000 per quality-adjusted life years) of 57.5% and 42.3%, respectively.