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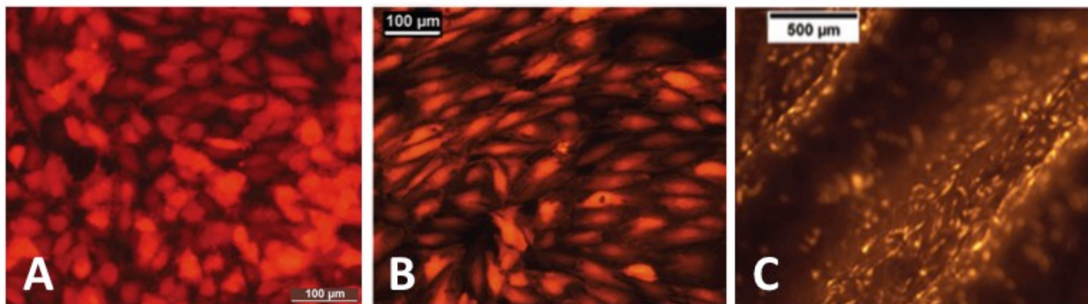
Research Interests:

Isolation, labeling and qualification of endothelial progenitor cells in tissue engineering.

My interests have been centered on bone or vascular tissue engineering. Vascularization or endothelialization remains one of the main hurdles that must be overcome to reconstruct tissues, even with the help of biomaterials and autologous stem cells. Among the strategies to create vascularized scaffolds for tissue engineering is the prevascularization of the scaffold by an association with endothelial cells that could enhance angiogenesis and bone regeneration. Thus, vascularization is the key challenge in tissue engineering. For this purpose, stem cells and progenitor cells may hold the key to repair and could be used for tissue engineering and regenerative medicine in a wide variety of disease states. More precisely, the isolation of endothelial progenitor cells from human blood generated great hope because of particular benefit when compared with mature endothelial cells. We isolated endothelial progenitor cells from cord blood and differentiated into endothelial progenitor cells (EPCs). However, the location of endothelial cells in a 3D matrix raises question of traceability. Also, the potential effectiveness of stem cell therapies is dependent upon homing of transplanted cells to relevant target organs and in this respect cell tracking appears to be a critical feature. To fulfill these requirements, cell fluorescent labeling by lentiviral transduction is a method used to track cell location and distribution within a matrix. We chose lentiPGK-TdTomato transduction for cell labeling because TdTomato fluorescent protein offers an exceptionally bright red fluorescent protein. We evaluated: *in vitro* the influence of lentiPGK-TdTomato transduction with the aim of qualifying human labeled- EPCs for cell tracking experiments and we checked related potential applications of EPCs in the context of tissue engineering, cells being seeded on scaffolds , in static or under shear stress conditions. It is possible to label human EPCs with TdTomato without affecting the viability and capacities of these cells, suggesting no gross modification of the cell-specific

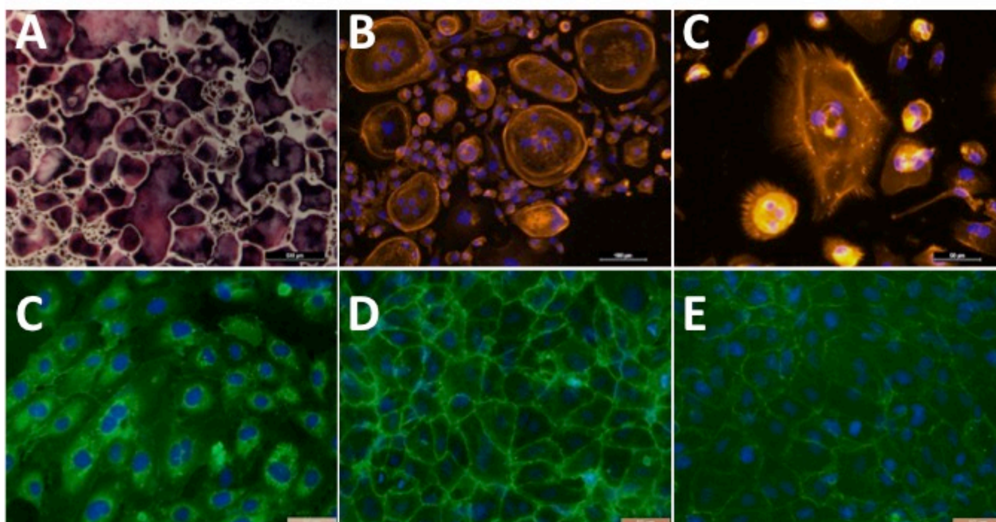
properties by the transduction procedure. This creates an important tool for numerous *in vitro* applications (1).



Microscopic appearance of the TdTomato-labeled EPCs, under static (A) and flow (B) conditions. Tubular 3D vascular prosthesis seeded with labeled EPCs (C).

Development of coculture model to investigate osteoclast/endothelial cell communication.

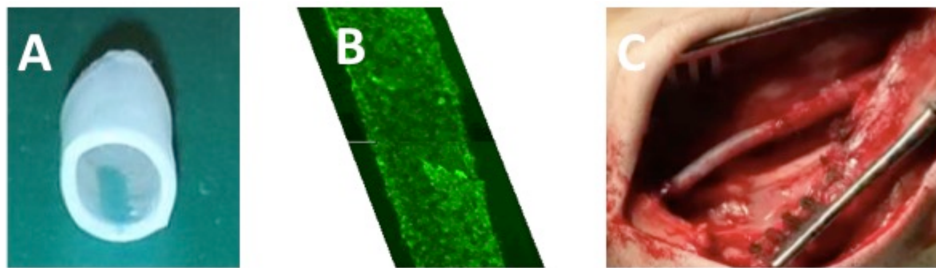
The critical role of vascularization in bone development, growth and repair has been well established. Bone is a living tissue mainly populated with osteoblastic cells, osteoclastic cells and endothelial cells. Their crosstalk is essential for bone homeostasis and to optimize bone regeneration after biomaterial implantation. *In vitro*, co-culture systems continue to increase our understanding of cellular communication. We developed *in vitro* co-culture system from human primary cells to investigate communication between osteoclast and endothelial cells. The challenge was to develop a co-culture medium without affecting the phenotype and capacities of these cells. Mononuclear cells were isolated from human umbilical cord blood. Osteoclast and Endothelial Progenitor Cells (EPCs) were obtained after differentiation. EPCs were used as endothelial cell model. Osteoclastic differentiation was characterized by TRAP staining and activity was assessed on calcium phosphate substrates. Osteoblast and EPC were characterized. In co-culture, EPCs and osteoclasts' phenotype and functional activity were maintained.



Osteoclasts characterization: TRAP staining (A) and actin ring (B) immunostaining
EPCs characterization: vWF (C), CD31 (D), Ve-cadherin (E) immunostaining

Development of a chitosan-based hydrogel small diameter vascular graft.

Vascular disease is the major cause of death in Western society. A common surgical treatment is to by pass blocked or narrowed artery with autologous vessels. However, the availability of autologous vessels is limited. Prosthetic grafts have been developed as alternatives. But, synthetic small diameter vascular grafts continue to disappoint because of thrombogenicity and the intimal hyperplasia. Consequently, there is strong clinical and societal demand for a novel biomaterial to produce small caliber vessels with better patency. We hypothesized that chitosan, a natural biomaterial, could provide a novel biological scaffold for vascular graft development. Chitosans are biocompatible natural polymers, investigated for a wide range of biomedical applications, among which tissue engineering. We developed chitosan-based hydrogel vascular grafts (in collaboration with the laboratory IMP in Lyon). The mechanical properties were assessed by measuring burst pressure, suturability and young modulus.
We investigated their biocompatibility focused on hemocompatibility, a prerequisite and essential property for vascular engineering. *In vitro* studies established endothelial cell coverage of chitosan and no blood component activation. *In vivo* studies showed no chronic inflammation in rat and satisfactory short-term hemocompatibility in sheep. Initial performance of a chitosan-based vascular graft is promising and future experiments will be undertaken to evaluate long-term patency.



Elaboration of chitosan-based vascular graft (A), colonized by EPC in vitro (B) and implanted as carotid artery interpositional graft in sheep (C)

Keywords/expertise:

- Tissue-engineering
- Vascular graft
- Cardiovascular biology
- Endothelium
- Chitosan
- Human cell culture
- Cocultures
- Mechanical stimulation
- Histology
- Immunofluorescence

Selected publications:

NB. Thébaud, **A. Aussel**, R. Siadous, J Toutain, R. Bareille, A. Montembault, L David, L.Bordenave. Labeling and qualification of endothelial progenitor cells for tracking in tissue engineering: An in vitro study". Int J Artif Organs 2015.

Teaching Activities:

<u>**Lectures**</u>

- **In dental school of Bordeaux University**
  histology (*alveolar bone*), biochemistry (*cell/matrix interaction*)
- **In Engineer School « ENSCBP »**
  (*stem cells in vascular tissue engineering*)
- **In Master « Parcours Recherche Santé »**
  (stem cells in vascular tissue engineering, technological advances in vascular engineering)

<u>**Tutorials**</u>

Assistant for microbiology tutorials

Clinical Activities:

- Pediatric dental surgeon in University Hospital Center (CHU) of Bordeaux
- Investigator in Multicenter Trial Comparing One-step Partial Caries Removal to Complete Caries Removal for the Treatment of Deep Carious Lesions in Permanent Teeth. (DECAT: DEep CAries Treatment). (DECAT) This clinical trial is a national multicenter randomized controlled trial performed in parallel groups aiming to validate a treatment that preserves pulp vitality of mature permanent posterior teeth through partial removal of carious tissue and restoring tooth structure using a simple filling in one session, thus delaying premature tooth aging

Memberships:

SFOP (Société Française d'Odontologie Pédiatrique)
SCADA (Student clinicians - American dental association)
CNESBO (Collège National de Sciences Biologiques)
SFBD (Société Francophone des Biomatériaux Dentaires)

Awards:

2013 Winner of the ADF-DENTSPLY thesis prize for dental thesis entitled "Méthodes de suivi des cellules souches dans le cadre de l'ingénierie tissulaire : évaluation expérimentale d'une méthode de fluorescence utilisant la protéine td-Tomato "

2015 Winner of Michel Degrange grant