Development of immunoactive biomimetic collagen constructs for airway transplantation

Context

Airway transplantation remains a great surgical and biological challenge. This is still an unsolved issue for patients in therapeutic impasse because of major tracheobronchial lesions requiring surgical resection and airway reconstruction. Our collaborator Prof. Martinod (thoracic surgeon, Hôpital Avicenne) has shown that Cryopreserved Aortic Allograft (CAA) transplantation was efficient in inducing trachea regeneration with chondrogenesis [1]. *De novo* generation of cartilage was observed after a few months, reconstituting an alternate structure of cartilage rings and allowing stent removal after an average period of 18 months. Most interestingly, one patient had a rapid recovery of tracheal functions (removal of stent) within 7 months, while others had a slow recovery that lasted for up to 39 months [1,2].

Studies establishing that either the absence of inflammation or intense inflammation cause poor regeneration in animals led us to **hypothesize that local immune regulation could be key to tissue regeneration modulation and interindividual heterogeneity in airway transplantation in clinical outcomes** [3]. However, **to test this hypothesis requires a suitable material capable to perform the role of *i.* a structural replacement for the trachea and *ii.* that of a local immunomodulator.**

It has been established that interleukin 33 (IL-33), expressed by a wide variety of cell type, is instrumental to induce tissue FOXP3 expressing regulatory (Treg) cells. Recent findings [4] have highlighted their role in tissue regeneration, and the CIMI team has recently observed that CAA released IL-33 and that regenerating trachea are infiltrated by tissue Treg cells. CAA being a limited resource, the project also aims at developing alternative biological devices that can promote tracheal regeneration. Supervisor 2 recently developed a process allowing precision shaping of highly concentrated tubular collagen materials in non-denaturing conditions [5]. The topotactic fibrillogenesis strategy enables self-assembly of type I collagen into fibrillar constructs and, simultaneously, stabilization of the macroscopic patterns defined by the ice templating technique. It therefore enables to control the porosity, which favors cell colonization, and to reproduce the features of the extracellular matrix (ECM), which is key for mechanical properties and cell adhesion. In addition, the elaboration process allows for the encapsulation of immunomodulatory molecules such as cytokines, opening a pathway to create immunoactive scaffolds that reproduce the relevant mechanical, textural and biological aspects of the native tissue.

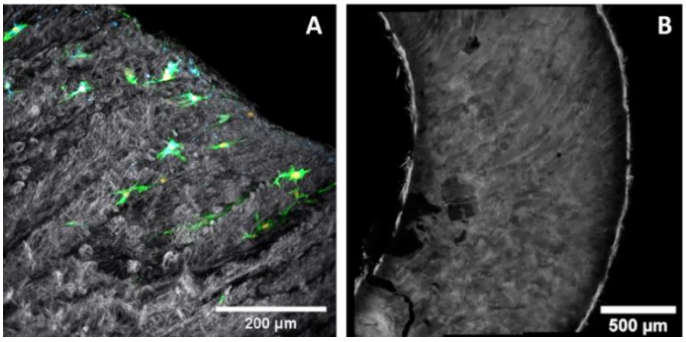

Figure 1. Collagen-based macroporous materials produced by ice templating followed by topotactic fibrillogenesis. A) Confocal microscopy (z projection) of a flat collagen matrix being colonized by Normal Human Dermal Fibroblasts (NHDF). Gray corresponds to fibrillar type I collagen observed by Second Harmonic Generation (SHG), green codes for actin (phalloidin), red codes for the nuclei (TO-Pro) and blue codes for cell proliferation (Ki-67). B) Confocal imaging (SHG) of a small diameter fibrillar collagen tubular construct.

Objectives and research hypotheses

We hypothesize that collagen bioprotheses that can be loaded with immunoactive biomolecules provide an ideal vector to study and to control airway regeneration. The biomimetic collagen constructs plays two roles. That of a biomimetic replacement for the currently used grafts with low biological noise—as opposed to the multitude of biomolecules and cells present in CAA—and that of a local immunomodulator delivery system. Based on the observation that CAAs currently in use release IL-33 at concentrations close to those observed to induce tissue Treg cells in mice [4], and that tissue

Treg cells are prevalent in regenerating trachea led to the **secondary hypothesis that local IL-33 dependent Treg cells may accelerate tracheal regeneration**.

The project aims at elaborating bioengineered scaffolds with optimized properties: i. recapitulation of the architecture of the aortic extracellular matrix, ii. ability to encapsulate and release immunoactive biomolecules such as IL-33 and other cytokines involved in the regeneration mediated by Treg cells, iii. ability to load and vectorize Treg cells.

The ultimate purpose of the project is to optimize local concentrations of IL-33 to enhance Treg mediated tissue regeneration and accelerate tracheal repair. The ice templating technique further offers the possibility to adjust the compositional, mechanical and geometrical properties of the scaffolds in order to optimize potential surgical protocols. Furthermore, recent results from the LCMCP have proven that ice templated collagen scaffolds enhance drastically cell migration, colonization as well as their 3D morphometric parameters, comforting their relevance in mimicking cell-matrix interactions [6].

Research plan

Task 1 Optimisation of bioengineered scaffold fabrication (Leader Fernandes) - Based on previous work developed at the LCMCP, two tubular construct fabrication setups will be built. A setup for the fabrication of flat versions of the collagen constructs will be developed in parallel to ensure *in vitro* colonization experiments can proceed in standard cell culture conditions. The transposition of the tubular to flat fabrication setups builds upon previous models developed at the LCMCP. The main levers for morphological control of the scaffold are ice front velocity, collagen concentration and thermal conductivity of the mold parts. These aspects will be considered when transferring the tubular to flat molds.

The bioengineered scaffolds consist on collagen tubes elaborated by ice-templating followed by topotactic fibrillogenesis according to a modification of the method reported previously [5]. The velocity at which the collagen solutions are plunged in the liquid nitrogen and the thermal conductivity of the mold parts determine the kinetics and orientation of ice growth, allowing to control the size and the morphology of the pores inside the tubular constructs. The porosity plays a critical in defining the access of cells within the scaffold's walls. In addition, porosity control translates into mechanical properties that can be modulated. Data at low internal pressures in small diameter equivalents of tubular matrices have provided elastic moduli of 77 kPa (comparable to 120 kPa for canine arteries under equivalent internal pressure). The mechanical characterization will here be implemented on an apparatus developed by the LCMCP team. The feedback from the materials characterization techniques (SEM, TEM, confocal microscopy, and mechanical characterization) will define four optimal fabrication conditions (2 porosity ranges and 2 elastic moduli ranges).

Task 2 Cytokine and Tregs loading in collagen matrices (Leader Fernandes) - Transforming the collagen matrices into immunoactive devices is divided into two sub-tasks.

2.1 IL33 loading on collagen matrices. Preliminary results (LCMCP & CIMI) have shown the ability to encapsulate the immunomodulatory biomolecules (IVIG and PEG-interferon) in collagen matrices during the materials' fabrication. Moreover, these materials can release such molecules for at least 6 days. We will encapsulate IL-33 during the ice templating stage to load the collagen matrices with two distinct cytokine concentrations. These results will be compared with those obtained by the cytokine impregnation of the collagen tubular matrices from IL-33 solutions. The loading method will be selected based on the sustained release of the cytokines in buffered aqueous media, detected by SIMOA or ELISA (according to the required detection limit). Upon selection of loading strategy, IL-33 loaded and collagen only tubular matrices will be assembled into tubular materials composed of alternating rings of IL-33 loaded and collagen to reproduce the tracheal segmental arrangement.

2.2 Treg loading of IL-33 containing collagen flat matrices. IL-33 rich matrices (collagen-only as control, homogeneous IL-33 spatial distribution and alternating IL-33 spatial distribution) will be colonised by Tregs in standard cell culture conditions. Tregs diffusion and colonization of the collagen

matrices will assessed using confocal microscopy (vital dye stained Treg cells) and Second Harmonic Generation (collagen) up to 7 days to rationalize the colonization success based on local IL-33 concentration. Treg cell density and special arrangement in the collagen matrices at days 1, 3 and 7 will be considered the defining success criteria.

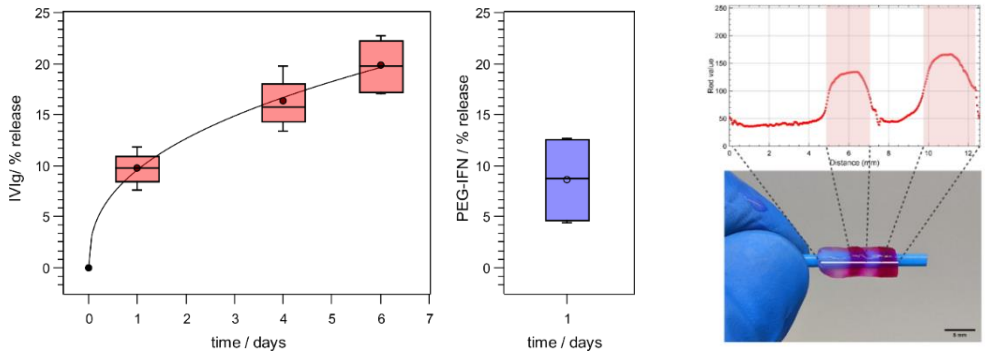

Figure 2. Left: Efficient encapsulation of IVIG and PEG-interferon *in vitro* release profile from macroporous collagen scaffolds at 37 °C: IVIg over 6 days and Pegylated-IFN at 1 day. Right: Periodic concentration profile of a model dye molecule in tubular collagen gels. Top image: red channel integration of the periodic tubular matrix.

Task 3 *In vitro* analysis of cellularization of immunomodulatory collagen matrices (Leader Miyara) - The interaction of the immunomodulatory collagen matrices (IL-33 & IL-33+Tregs) with the surrounding cellular environment of the trachea will be studied using flat matrices. Human bronchial epithelial (HBE) cells will be seeded and cultured on the inner side of immunoactive or non immunoactive collagen matrices (4 matrices per group) in order to test cell adherence, viability, proliferation and differentiation. HBE secretome will be analyzed to detect the release of molecules with potential adverse effect (inflammation and bronchoconstriction).

Task 4 *In vivo* evaluation of the matrices (Leader Miyara) - We will study direct/indirect interactions of Tregs with chondrocyte progenitors and their role during tracheal regeneration in B6 mice. Immunomodulatory collagen matrices (IL-33 & IL-33+Tregs or empty matrices) and blood will be harvested on day 7, 21 and 60 after surgery (total n=90 mice, 10 mice for each point). First, foxp3 expressing cells will be traced *in vivo* using transgenic mice (foxp3-EGFP) to localize and identify their effects on Immunomodulatory collagen matrices (allograft survival, balance pro/anti-inflammatory cytokines, chondrocyte proliferation, differentiation, migration, ECM production). Second, the ability of IL-33 to influence chondrogenesis will be assessed *in vivo* in transgenic mice deleted or not for *Il-33* or *St2* genes (total n=60 mice, 10 mice for each point). Effects of Tregs on Immunomodulatory collagen matrices will be assessed by 3D immunohistology and multiplex analysis.

Outcomes and transfer potentiality

Through this project we aim at demonstrating the ability of immunomodulatory collagen matrices to deliver IL-33 and Treg cells *in vitro* and to accelerate neochondrogenesis and tracheal regeneration in pre-clinical models. These results will set the basis to move forward to pre-clinical validation in big animals and to first in man clinical trials. The macroporous collagen matrices developed by the LCMCP are currently being considered for patentability by the SATT Lutech. Its protection as drug delivery devices will be considered during the 1st year of the PhD project.

References

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