The CABMM'S Professors Stephen Ferguson and Karin Wuertz-Kozak on the potential of electrospinning for regenerative medicine and tissue engineering

Electrospinning for regenerative medicine and tissue engineering

The use of biomaterials for the substitution of natural tissues and the restoration of biomechanical function is well established in medicine, enhanced by constant improvements in the formulation and application of such materials over the past century. While the emphasis was initially on establishing bio-inert, long-lasting implants with a proven ability to survive for years, if not decades, in the body, the potential for using biomaterials to support and guide the body's own regenerative potential has come into focus in the past two decade.

Profound advances in our understanding of a natural tissue's biochemical and micro-architectural composition have driven the development of biomaterials down the path towards truly biomimetic concepts. Natural tissues are characterised by heterogeneity on the macro-, micro- and nano-scales, complex multi-phasic constituents, fibre-reinforcement and a direction-dependent organisation of the extracellular matrix in response to functional demands. Furthermore, the interaction between the cell and its surrounding matrix is strongly influenced by the local pericellular chemistry and topography, providing clues for the design of improved functional biomaterials.

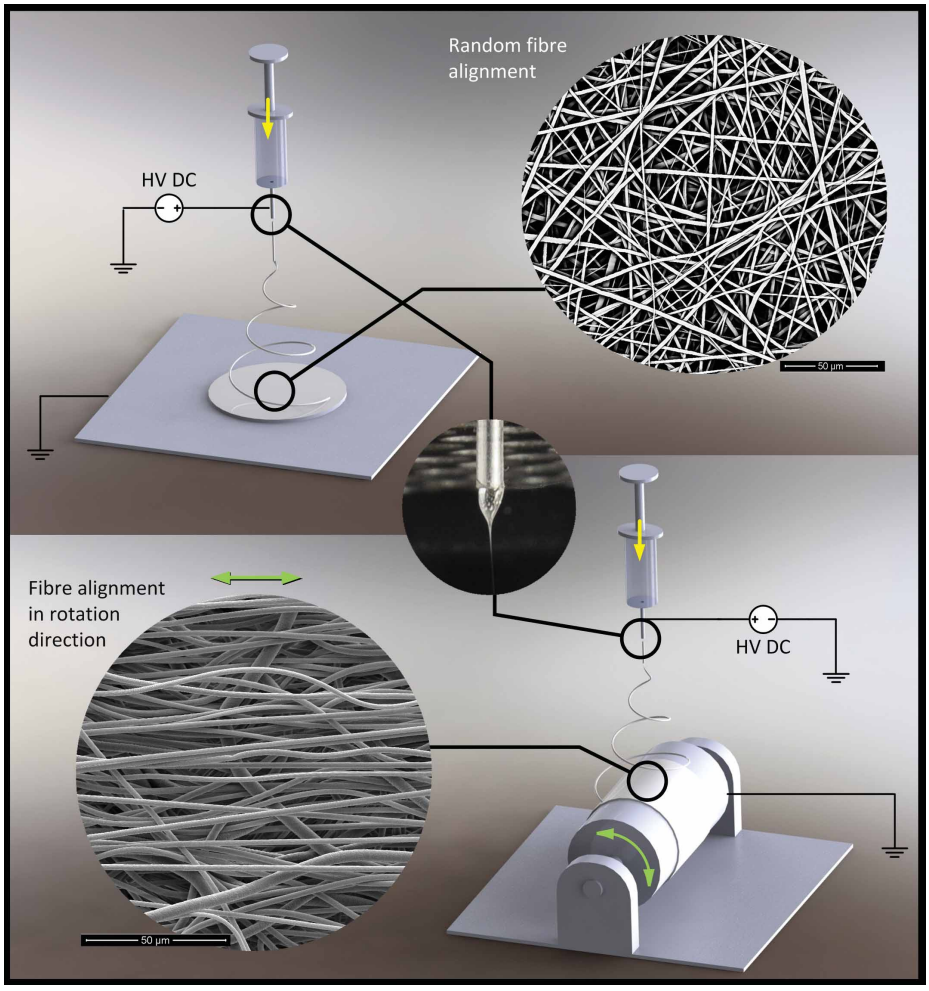

Fig. 1 Schematic illustration of the electrospinning process to create flat membranes. In the most common set-up (top), the charged polymer solution is drawn from the tip towards a grounded plate, onto which the fibers arrange randomly. In order to create aligned fibers, a grounded rotating mandrel can be used (bottom)

Biomembranes

Biomembranes are central structural elements throughout the body, for example in blood vessels, the heart, the bladder, the liver capsule, tendon sheaths, spinal discs and skin, to name just a few. Early attempts to use synthetic membranes for their repair were often limited to as-cast polymer sheets, which – while often compliant enough – exhibited a homogenous, isotropic composition that did not fully capture the unique mechanical response of these biomembranes. Furthermore, such uniform membranes often provided no means for early cell integration.

An important advance in the creation of biomimetic membranes was achieved through textile production methods, combining polymeric or even natural fibres into complex interlocking arrangements using weaving or stitching methods. Numerous concepts for textile implants can be found in the patent literature, with several relevant devices already reaching clinical application, e.g. vascular grafts and hernial repair meshes.

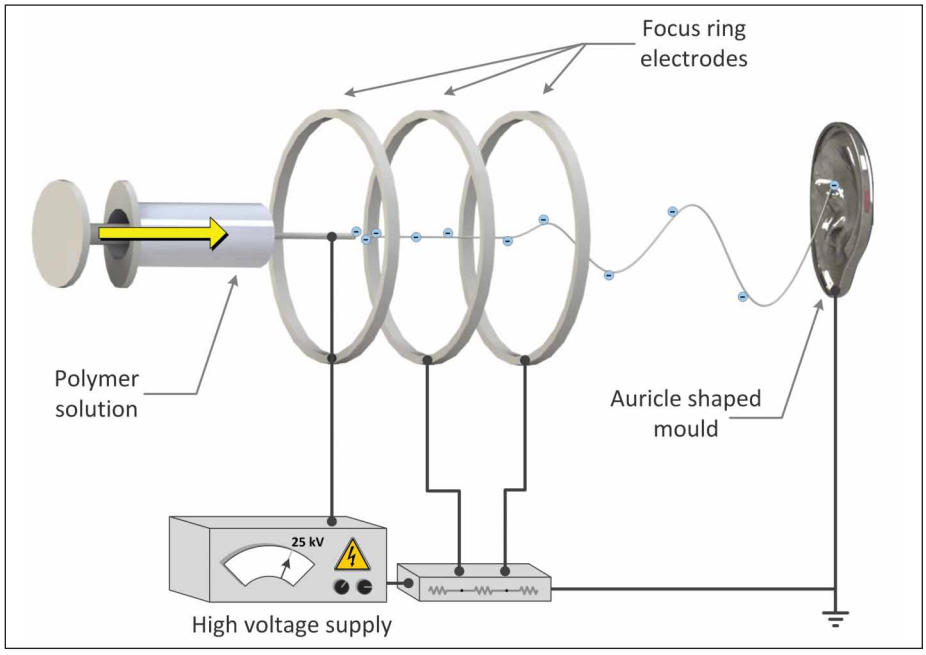

Fig. 2 Schematic illustration of the electrospinning process to create ear-shaped scaffolds. The syringe needle and the first auxiliary ring electrode are connected to the high voltage potential. The two other ring electrodes, whose electrical potential can be controlled, create a precise electrical field that allow controlled fibre deposition on the ear-shaped stainless steel mould

While textile implants fulfill the overall mechanical demands of a biomembrane and are suitable for long-term implantation, they are less applicable for regenerative medicine applications, whose success strongly depends on the cells' responses to the biomaterial structure and hence its topography. Thus, a concentrated effort has been made in the last decade to develop new methods for the production of nanofibrous biomaterial structures that most closely resemble the natural extracellular matrix of the target tissue. Of these, the process of electrospinning is particularly promising.

How does the electrospinning process work?

The electrospinning process (see Fig. 1) makes use of electrostatic repulsion in the production of discrete fibres from polymer solutions. High voltage applied to the droplet of a viscous polymer solution emerging from a small-diameter tube or syringe induces a charge on the liquid. Electrostatic repulsion draws the fluid into a 'Taylor cone', from which a fine jet is propelled towards a grounded collector. During the flight of this jet, the solvent evaporates and a whipping action in the jet further stretches and thins the fibre.

The final characteristics of the electrospun fibre mats (fibre diameter, fibre alignment, porosity, pore diameter) depend on a large number of parameters related to the polymer solution (solvent choice, temperature, polymer weight, polymer percentage, viscosity), the applied electrical field (voltage, electrode positioning), the local environment (temperature, pressure, humidity) and the nature of the collector. The versatility of electrospinning, in combination with the ability to create tailored biomembranes fulfilling the explicit demands of selected applications, explains the growing interest in this technique.

Over the past decade, the potential of electrospinning has been demonstrated for the creation of a variety of nanofibrous membranes for both consumer products (e.g. semi-permeable membranes for functional clothing) and biomedical devices (e.g. membrane for the repair of vascular tissue, intervertebral disc, meniscus, tendon, ligament and hernias), some of which have been extensively studied in our own groups.

Reconstructive procedures for the ear, nose and throat (ORL)

Patients who suffer from congenital deformities of the auricle, or have lost parts of the organ due to trauma or tumour resections, require reconstructive procedures in order to ensure their quality of life. Reconstruction not only provides functionality (i.e. hearing), but ideally also serves an aesthetic purpose, hence allowing patients to maintain or regain a self-assured behaviour, especially when interacting with others.

The current gold standard for auricular reconstruction is harvesting autologous cartilage grafts from, for instance, the rib cage, which – after being manually shaped by the surgeon – are implanted to replace the lacking tissue. However, this treatment has a substantial risk for donor site morbidity, can cause undesired scarring, and is generally limited by the availability of sufficient cartilage material.

In order to overcome these limitations and reduce risks and complications, effort has been put into the development of tissue-engineered implants, e.g. by using electrospun membranes. While electrospun membranes possess mechanical strength similar to that of the native tissue, and a microstructure mimicking the natural nanofibrous structure, their geometries are usually limited to either sheet-like or tubular structures.

We have been able to demonstrate the feasibility of electrospinning fibrous scaffolds directly into an auricle-shaped conductive mould in order to provide a form that is close to the desired

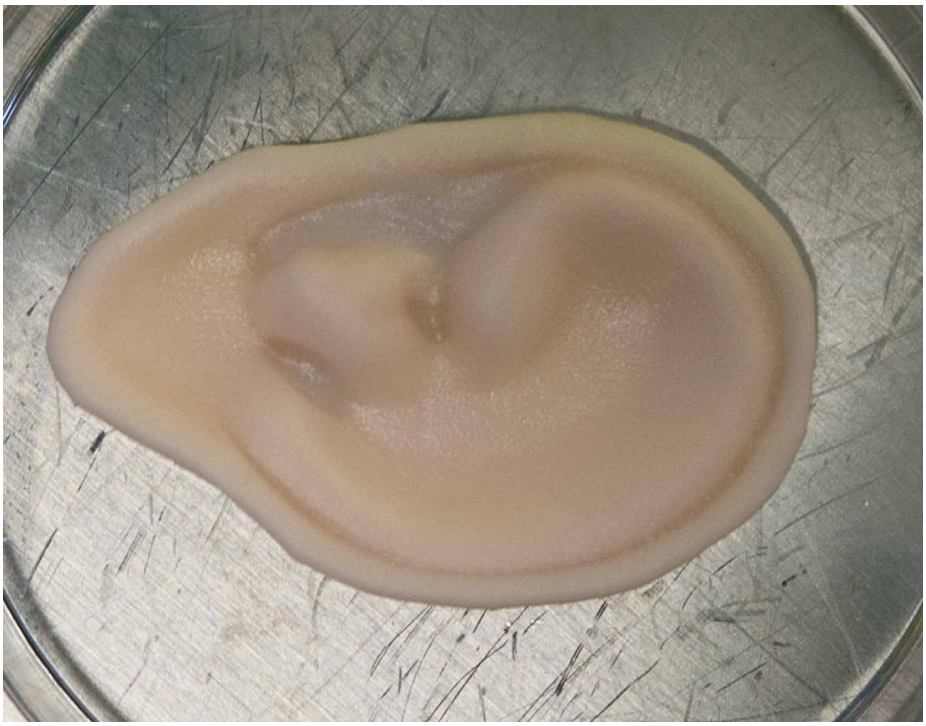

Fig. 3 Electrospun ear seeded with bovine chondrocytes after two weeks of culture

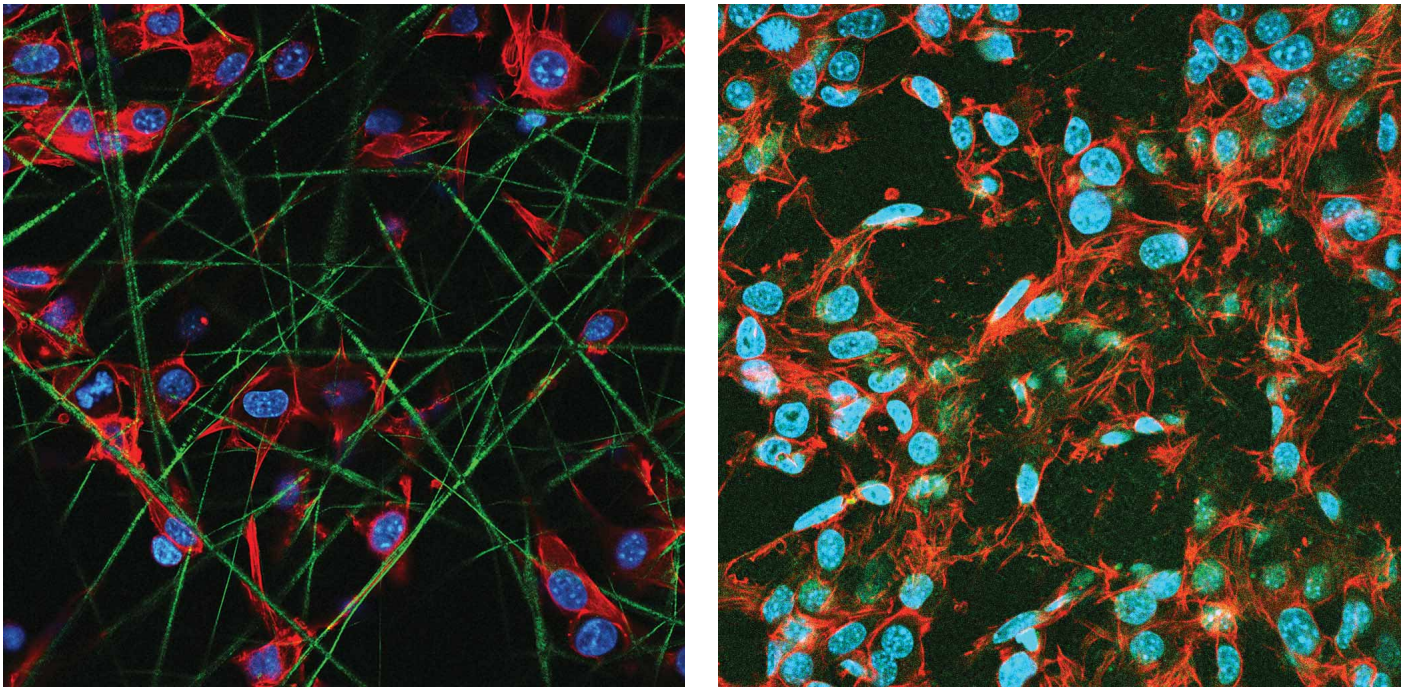

Fig. 4 Confocal microscopy images of 3T3 cells 24 hours after seeding onto the double-layered, electrospun membrane of the AF repair system. On the left, the cell-adhesive PCL side is depicted, whereas the right picture shows the cell-repellent PVDF side. Red: Phalloidin staining of the cytoskeleton; Blue: DAPI staining of the nucleus; Green: Autofluorescence of fibres

auricular shape and requires minimal further processing (see Fig. 2). These poly(ε)caprolactone (PCL)-based constructs were assessed for their potential in cartilage tissue engineering. Ear-shape constructs were seeded with primary chondrocytes and the regional biochemical and biomechanical response as well as their change over time in culture were investigated.

The 3D auricle-shaped constructs were successfully spun into the mould with a thickness of approximately 1mm, which corresponds to the thickness of native auricular cartilage of the human ear (see Fig. 3). They showed an excellent initial stability, returned to their shape after being folded or deformed manually and maintained their shape during the whole cell culture period. The chondrocytes seeded on the constructs showed rapid proliferation and extracellular matrix production. Importantly, a significant increase in the content of total collagen could be detected in all regions of the constructs. Stress-relaxation indentation testing revealed instantaneous moduli close to the values of native tissue.

This study demonstrates the feasibility of electrospinning fibrous scaffolds directly into an auricle-shaped conductive mould and hence underlines the potential of electrospinning for cartilage tissue engineering. Although good results were obtained, various approaches can be employed to further optimise the properties of these constructs: increasing the pore size, e.g. via incorporation of sacrificial fibres or use of cryogenic electrospinning, will enhance cell infiltration, whereas fibre alignment strategies can enhance the mechanical stability. Ultimately, these optimised scaffolds may find their way into the clinics as an alternative to autologous cartilage graft implantation.

Annulus Fibrosus (AF) repair

The intervertebral discs are located between the vertebrae of the spine, providing mobility during daily activity. Early in life, disc degeneration can occur, with a loss in water content in the centre (Nucleus Pulposus, NP) and reduced structural integrity of the outer ring (Annulus Fibrosus AF). Consequently, the gelatinous NP can bulge through areas of structural damage in the AF and irritate spinal nerves.

This condition, which is called 'disc herniation' (or also disc prolapse, or slipped disc), is one of the most common causes of lower back pain, as well as leg pain (sciatica), with very high costs for the society. If conservative treatment fails, two main types of surgical treatment are performed today:

- Removal of the herniated tissue (minidiscectomy); or
- Removal or the entire intervertebral disc (total discectomy) either as a fusion or total disc replacement approach.

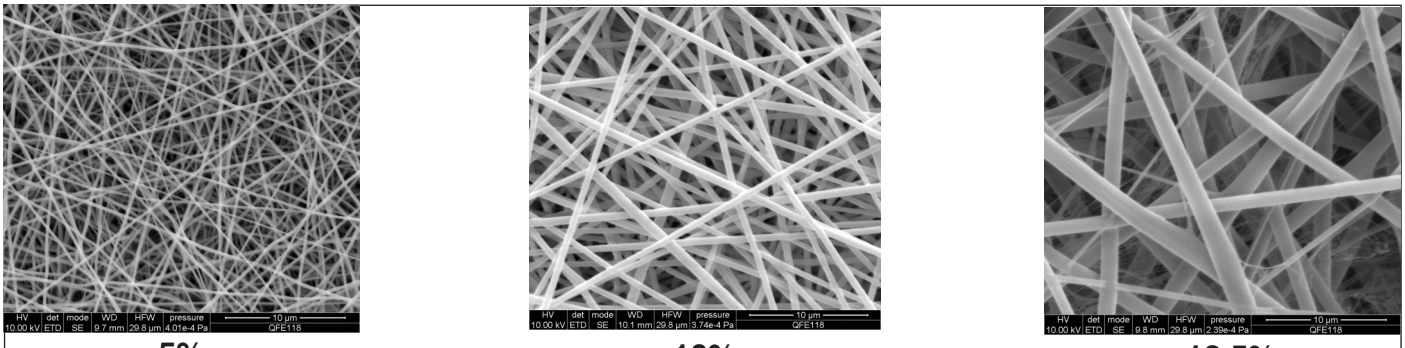

Fig. 5 Controllability of fibre diameter and pore size by alteration of the concentration of the polymer solution. This allows the production of tailored membranes suitable for specific applications

While minidiscectomy holds a large risk for reherniation, total discectomy tends to cause spinal instability, indicating that there is a clear need for more satisfactory new treatment options.

A functional AF repair system

By producing electrospun, multi-structured scaffolds, we aim to create a novel, tailored AF repair system that reduces the amount of native tissue that has to be sacrificed, causing immediate closure of the defect with sufficient mechanical strength and enables biological repair. Importantly, a functional AF repair system should consist of an inner cell-adhesive side to fulfil the abovementioned needs, but should also contain a non-adhesive outer side to prevent cell ingrowth of surrounding neural structures and the dura mater spinalis. The focus of this project is hence to create a bilayered membrane by electrospinning of two different polymers: PCL (polycaprolactone) and PVDF (polyvinylidene fluoride).

PCL, whose fibres closely resemble the collagen structure of AF if fibre alignment techniques are utilised, is used for the adhesive, inner side of the repair system. As seen in previous studies, cells show excellent attachment and migration on the electrospun PCL side, with alignment of the cell's stress fibres of the cytoskeleton to the PCL fibres. Furthermore, the slowly degradable PCL provides suitable mechanical stability that allows the bilayered implant to withstand physiological levels of strain (<20%).

The mechanical behaviour of the membrane is currently optimised by adjusting fibre alignment, regularity, fibre dimensions, inter fibre connections and crystallinity. First results indicate that the subsequently-produced electrospun networks exhibit inelastic behaviour during initial loading, followed by viscoelastic hysteresis during dynamic loading. In order to better describe, simulate and ultimately avoid mechanical failure of the membrane, 3D deformations within the membrane upon loading are observed by multi photon microscopy.

A non-adhesive, outer side is created by electrospinning PVDF directly on top of the PCL membrane. Electrospinning of PVDF creates a fine fibre mesh, with fibres being more than 20x smaller in diameter than PCL fibres (1.24 ± 0.6 µm *versus* 54.7 ± 0.02 nm). Cell culture studies with fibroblasts (3T3 cells) and nerve cells (NC-1 cells) demonstrated largely restricted adhesion, spreading and consequently migration and proliferation (see Fig. 4). In order to further enhance the cell repelling potential of the PVDF membrane, various biological modifications were tested. Infiltration of membranes with specific polysaccharides showed beneficial effects by further reducing 3T3 cell adhesion and maintaining non-adhesiveness for NC-1 cells.

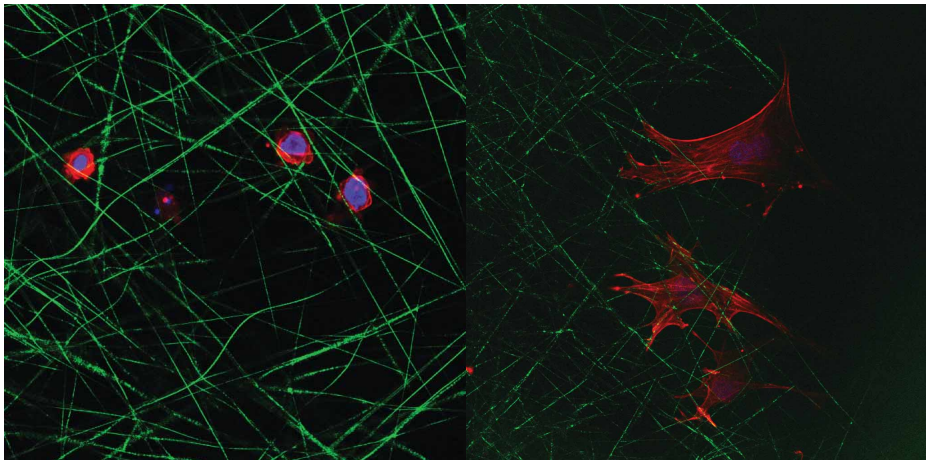

Fig. 6 HUVECs seeded onto normal electrospun membranes (left) or onto electrospun membranes with cell-specific coating (right). The coating improves cell attachment by supporting cytoskeletal adhesion to the polymer fibres. Red: Phalloidin staining of the cytoskeleton; Blue: DAPI staining of the nucleus; Green: Autofluorescence of fibres

In summary, a novel AF repair system consisting of bi-layered electrospun membranes with a (inner) cell-adhesive side (PCL) as well as a (outer) non-adhesive side (PVDF) could be fabricated that demonstrates biological functionality. As different cell types seem to respond similarly when being seeded onto the two sides of the membrane, this development may in fact have more clinical applications than only AF repair during spinal surgery.

Biomimetic blood propulsion

As illustrated in the first two examples, the most common approach to create tissue constructs with electrospinning is to infiltrate cells into a biodegradable scaffold made of polymer fibres. A less explored application of the electrospinning method is to create spun surfaces or membranes for culturing cells in monolayers. This approach gives the possibility of creating cell layers for various applications, such as tissue engineering of skin or endothelialisation of tissue engineered organs or devices.

The approach of using spun membranes for monolayer culture is currently being applied by our groups in the context of the 'Zurich Heart Project'. The goal of this project is to generate a highly deformable hybrid membrane that consists of a synthetic substrate covered by an endothelial cell layer. This bio-composite material system will integrate a living biological layer to form the basis of a 100% haemocompatible blood pump. For this application, the hybrid membrane is required to resist cyclic deformation and shear stresses from blood flow. Ensuring long-term integrity and functionality of the endothelium attached to the highly deformable substrate exposed to flow represents the major challenge of the new system.

We believe that the long-term functionality of the membrane, and hence the blood pump, might be achieved by creating a non-degradable layer of synthetic, electrospun fibres, mimicking the ultrastructure of natural biomembranes, that fully supports the adherence and function of the endothelium.

The development of the fibrous membrane involves the optimisation of the thickness, alignment and pore size of the fibres as well as use of surface coating that promotes cell attachment. Simply by adjusting the ratio of polymer in the spinning solution, it is possible to tune fibre thickness and pore size (see Fig. 5). While relatively large pore sizes are sought for those tissue engineering applications that require cell infiltration into a scaffold, relatively small pore sizes are pursued for endothelialisation. While initial endothelial cell attachment can be improved by suitable surface coating (see Fig. 6), biological modifications of the membrane through incorporation of specific natural or pharmaceutical compounds may enhance long-term functionality of the created endothelium.

Within the large context of the Zurich Heart Project, creating an endothelialised membrane with long-term functionality opens the door for a variety of cardiovascular applications, ranging from vascular grafts to biomimetic heart valves.

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http://www.cabmm.uzh.ch/en.html