The cornea, the transparent “window” at the front of the eye, must remain perfectly transparent to focus light and ensure sharp vision. However, the clarity of the cornea can be lost due to several issues, leading to vision loss or blindness. One of these issues is related to the inner layer of the cornea, namely the corneal endothelium. These cells act as a continuous pump, constantly removing excess fluid that the cornea naturally absorbs (like a sponge). The buildup of fluid in the cornea is prevented by the pump function of the corneal endothelium. But if to many of these pump cells die (due to hereditary diseases or as a complication of eye surgery), the remaining cells are no longer able to prevent swelling, resulting in the cornea turning cloudy, and ultimately blindness. These cells cannot regenerate naturally in humans, so currently the only treatment is a corneal transplant. However, there is a global shortage of donor corneas. This encouraged researchers to find alternative strategies which are less dependent on donor tissue. One of these strategies involves the delivery of nucleic acids towards these corneal endothelial cells, which could temporarily induce cell proliferation. If these cells can temporarily grow, the total number of pumps present on the endothelium would increase, thereby preventing fluid buildup in the cornea. In addition, nucleic acids could also be employed to prevent these cells from dying in specific conditions, or treat the genetic mutation in case of hereditary diseases. However, these nucleic acids are significantly bigger than standard drug molecules. Therefore specialized techniques to deliver these nucleic acids into the cells are required. This thesis focusses on the development and discussion of several of these techniques for the corneal endothelium.

Chapter 1 summarizes all the delivery techniques which have been explored for the delivery of nucleic acids to the corneal endothelium. Here, the techniques are divided into physical techniques, which create a transient hole in the cell membrane through which the nucleic acids can enter the cell, or biochemical techniques, which form ‘packages’ with the nucleic acids to facilitate cellular uptake. Each of these techniques has several benefits and drawbacks which are discussed, alongside their potential for future applications.

Chapter 2 explores the performance of DOTAP:DOPE lipoplexes, a biochemical technique, against commercially available lipid-based transfection kits for the delivery of nucleic acids to corneal endothelial cells grown in a dish. In addition they were also tested on endothelial cells present on a bovine cornea, serving as a more complex model. While these commercially available lipid-based transfection kits are often efficient, they are not suitable for applications in a clinical setting. Therefore, DOTAP:DOPE lipoplexes are explored as a clinically translatable alternative.

Chapter 3 investigates a novel physical technique called photoporation. Here transient pores are created in the cell membrane by applying very short (several billionths of a second) laser pulses on gold nanoparticles which are in close proximity to the cell membrane. The performance and safety of this technique will be evaluated on corneal endothelial cells grown in a dish, but also on corneal endothelial cells present on bovine- and even human corneas. Lastly, we will also explore its performance in living rabbits. In order to do so, we have embedded these gold nanoparticles, together with the nucleic acids, inside a hyaluronic acid gel. This gel can then easily be injected into the anterior eye chamber to coat the corneal endothelium. After laser irradiation this gel can be removed. This is very similar to a procedure employed during cataract surgery to protect the corneal endothelium from damage.

Chapter 4 focusses on another cause of corneal blindness, namely microbiological infections of the cornea. More specifically, it focusses on a particularly devastating infection caused by *Acanthamoeba*. During the infection phase, these *Acanthamoeba* are present in their active life form, 'eating' their way through the cornea. However, when confronted with medication or a hostile environment, they transform into a dormant life form with a protective double shell. This life form is extremely resistant to drugs and hard to eradicate, often resulting in intensive and long treatments. In this chapter we will explore a novel physical mechanism to specifically destroy this double walled dormant state. More specifically, we create a powerful mechanical force that shatters the cyst wall, thereby killing the parasite.

Lastly, **Chapter 5** situates the findings described in this thesis within the broader international context. It also discusses how nucleic acid delivery techniques for the corneal endothelium can help in the treatment of corneal blindness in the future. In addition several challenges still need to be addressed before these strategies can be implemented into clinical practice, including regulatory and economical challenges.