

## 2.2 Eye banking; developments

### Period when living donor tissue was used (1906-1935)

For the first successful human graft, performed by Zirm from Olmutz in Slovakia in 1906, freshly harvested tissue was used from an 11 year old living boy, who lost vision because of an intraocular foreign body (see chapter 2.6). The eye was kept in warm physiological saline solution and the transplantation started without delay. After the graft had been cut, it was held between two pieces of saline moistened gauze over a steaming container of hot, sterilized water. Zirm believed his careful handling of the tissue contributed to the successful outcome.<sup>75,76,77</sup>

During the first half of the 20th century, fresh donor tissue was considered of paramount importance to the success of a corneal graft. Transplantation was performed using tissue from enucleated eyes from living donors and the graft was retrieved and judged by the corneal surgeon himself.<sup>78</sup> This procedure was effective even in its earliest stages of development. In the Netherlands living donors were the only source for donor corneas in the period 1939-1946.<sup>79,80</sup> Between 1976 and 1980 living donors, diagnosed with malignant choroidal melanoma, made it possible to start with tissue typed and matched corneal grafts. In those days the typing of blood from living donors, was more reliable than the typing of cadaveric donors.<sup>81,82</sup>

### Period when post-mortem tissue was used (1935- today)

The father of modern eye banking was Filatov from Odessa in the Ukraine. He reported in 1935 the transplantation of 35 corneas obtained from human cadaver eyes, collected within a few hours after death.<sup>83,84</sup> His storage method was essentially the same as the moist chamber technique still used by many ophthalmologists throughout the world today (see chapter 2.5).<sup>77,78</sup> The proven suitability of post mortem tissue has had a great impact on the frequency of grafting due to the availability of a larger source of donor tissue.

### Period of experimental storage methods for corneoscleral discs (1960s-today)

To reduce autolysis of the corneal endothelium in the cadaver eye methods for handling the excised corneoscleral discs were studied.<sup>85</sup> One idea was to store the corneoscleral disc in serum.<sup>78,86</sup> Another idea, in particular to obtain a long-term storage, was cryopreservation. The first clinical success with this technique was claimed by Eastcott.<sup>87</sup> In the following years the technique has been developed.<sup>88,89</sup> Concentration of cryoprotectants, the rate of freezing during different phases of the process and the velocity of thawing seemed to be critical factors but up till now no method has been found to protect the corneal endothelium from freezing damage.<sup>90,91</sup> Despite its promising advantage of indefinite storage time and reported clinical successes, cryopreservation has not become a routine storage method for full thickness grafts.<sup>92</sup> Another approach, lyophilisation (freeze-drying)

is currently advocated for corneal lamellae.<sup>93</sup> Cryopreservation and vitrification seem promising for storage of isolated cells.<sup>94</sup>

### **Period of storage of corneoscleral discs in tissue culture medium (1973-today)**

Cold preservation techniques and warm preservation techniques have been introduced in the same period in the 1970s and have both developed in their own way. The key difference is the storage temperature and its impact on corneal metabolism.

#### *Development of hypothermic storage solutions*

The introduction by McCarey and Kaufman in 1974 of a well defined tissue culture medium (TC 199 with 5% dextran) was a major step forward in the routine preservation. The excised corneoscleral disc was maintained in a culture medium.<sup>95,96,97</sup> On the one hand it made the excision of the corneoscleral button in situ possible, increasing the donor supply and on the other hand a storage time of 14 days was advocated.<sup>98,99</sup> Nevertheless in eye banking practice the generally accepted storage time is 72 hours at the most. Longer storage time became desirable for the surgical scheduling, both for the benefit of the surgeon as well as for the patient, and for gaining time for serology testing. Another formulation for the preservation medium therefore was sought. In Europe, some eye banks used Dispersa or Likorol for this reason.

Also a beneficial effect of chondroitin sulphate compared to dextran has been described.<sup>100,101,102</sup> In Japan chondroitin sulphate has been used in storage media since 1968.<sup>103</sup> K-Sol, a chondroitin sulphate containing medium, was introduced in 1985.<sup>100</sup> Because of bacterial contamination of the medium K-sol is no longer produced despite the good clinical results that have originally been described.<sup>104</sup>

The subsequent Dexasol aimed a better control of stromal hydration in vitro by supplementing the chondroitin sulphate with dextran. In addition, the original TC- 199 medium has been replaced by Eagle's minimal essential medium (MEM). Currently the most frequently used medium is Optisol (GS), a hybrid of K-Sol and Dexasol.<sup>77,102,105</sup>

Other components like vitamins, hydroxyprolin, growth factors and ATP precursors have been added since the introduction of Dexasol. These supplements were found to be effective in tissue cell culture. As preservation media were developed, the optimal antibiotic cocktail was searched for. Penicillin/ Streptomycin was used in McCarey Kaufman medium (MK), later on replaced by gentamycin (MK, K-Sol, Dexasol, Optisol) and finally by gentamycin/ streptomycin in Optisol GS. The concentration of the macromolecules and the composition of the dehydrating molecules (see chapter 2.4) aimed at an optimal corneal thickness for the manipulation during surgery.<sup>106</sup> With all these modifications a longer storage time is now claimed with a maximum of up to 14 days for Optisol (GS).

### *Development of organ culture storage procedure*

In organ culture the corneoscleral disc is preserved in tissue culture medium supplemented with bovine serum as a nutrient at a physiological temperature (30-37°C). At first, the primary goal was modification of the antigenicity to reduce immunological rejections,<sup>107,108</sup> but soon the advantage of the long time storage and the endothelium wound repair was more appreciated and the medium was developed for routine eye banking.<sup>109</sup>

In 1973 Summerlin was the first to store a cornea in organ culture medium in a petri dish for one month.<sup>107</sup> Doughman adapted the method for eye banking by changing from petri dishes to flasks as containers in 1976.<sup>108,110</sup> The tissue, swollen due to the absence of macromolecules in the storage solution, was hard to handle during surgery and it took a month for the graft to get clear.<sup>108</sup> Others reported poor results as well.<sup>111</sup> During the following years modifications were introduced. Doughman transferred the organ cultured cornea to MK at 4° C shortly before transplantation, but with this technique a significant higher post operative cell loss was reported.<sup>112</sup> Therefore, this development was discontinued.

To prevent corneal swelling Sperling added dextran T500, a macromolecule 10 times larger than the dextran used in the MK; he introduced this method in Europe in 1978.<sup>47,113</sup> Clear grafts were described but electron microscopy showed uptake of dextran in all corneal cells.<sup>114,115</sup>

In 1983 a modification of Doughman's and Sperling's method has been introduced by Pels; a first storage phase without dextran and a second short phase for reversal of the swelling in medium supplemented with dextran.<sup>113,116,117,118</sup> This offered a long-term storage and a cornea easy to handle by the surgeon. To exclude corneas that do not tolerate a long-term storage, evaluation of the endothelium is essential for a good performance of the organ culture procedure.

Sperling introduced an evaluation method for the corneal endothelium using light microscopy.

This way the organ culture procedure offers corneas with a well described endothelial quality. This was highly appreciated in a time that clinical endothelial evaluation was not the common procedure because the equipment, such as the specular microscope, was still in a development phase.<sup>119</sup>

### *Application*

Hypothermic storage is now used all over the world. The method in which organ culture preservation is combined with a transfer to a transport medium is the most widely used bank technique in Europe<sup>120,121</sup> and it is also used in Australia and New Zealand.<sup>122,123</sup> These are areas where the usefulness of HLA matched corneas has been studied and where HLA matched corneas are applied.

