The IACLE Contact Lens Course

MODULE 6

The Cornea in Contact Lens Wear

First Edition

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Acknowledgements

The IACLE Curriculum Project is the result of a desire to raise the standard of eyecare education, to make contact lens wear safer and more successful, and to develop the contact lens business by creating the educational infrastructure that will produce the teachers, students and practitioners of the future.

The concept of the world's best educators making available their most creative educational contributions for the common good without any recompense, other than a sense of satisfaction, was born out of IACLE's idealism.

The Curriculum Project could not be successful without the assistance and generosity of a large number of talented and dedicated people. To all those contributors of lectures, laboratory notes, videos, slides, etc., we say thank you. Your generosity of spirit will benefit many educators, hundreds of thousands of students and millions of patients throughout the world.

The Vice President of IACLE, Professor Desmond Fonn, has made a tremendous contribution since the inception of IACLE, and has provided his considerable expertise in the final editing stage of the Curriculum. This project was commenced under Professor Brien Holden's leadership. The original plan and layout for the Curriculum was prepared by Sylvie Sulaiman, IACLE's Director of Education. Sylvie's dedication and excellent understanding of practitioner and community requirements have given the Project focus and depth.

More recently, the IACLE Curriculum Project has benefited from the work of Dr Lewis Williams as Manager, Educational Development. Dr Williams has done an amazing amount of work to achieve an impressive collation of diverse material, and has created what I believe to be an invaluable collection of contact lens knowledge. Dr Williams has also been assisted by Rob Terry's considerable experience and understanding of the contact lens field.

Kylie Knox has done an excellent job as Project Editor. To complement the efforts of the editors, layout coordinators Barry Brown and Shane Parker have done an admirable job, as have the rest of the graphics team. The Cornea and Contact Lens Research Unit (CCLRU) at the University of New South Wales has contributed substantially to this project through the donation of time, resources and editorial support.

The IACLE global staff including Director of Administration Yvette Waddell, Global Coordinator Pamela O'Brien and Executive Secretary Gail van Heerden, have expertly managed the considerable tasks of production and distribution.

No acknowledgments page in an IACLE document would be complete without a reference to its sponsors. Bausch & Lomb have been a major corporate sponsor since 1990, providing the original stimulus for IACLE's growth by contributing financial support and the involvement of individuals from their International Division. It was Dr Juan Carlos Aragon (when he was at Bausch & Lomb) who first suggested that if IACLE was to be taken seriously by industry, it needed a global plan to address the educational requirements for the safe, effective growth of the contact lens business. Johnson & Johnson Vision Products is our other major corporate sponsor. They have provided excellent assistance through their provision of industry coordinators for Europe, Africa, and the Middle East. CIBA Vision has been a corporate contributor and also provided excellent industry coordination in Latin America. Allergan and Wesley Jessen/PBH have contributed generously as corporate donors, with Alcon Laboratories, contributing as IACLE donor.

IACLE is a cooperative effort, and none of its activities are more collective than the Curriculum Project. The IACLE Contact Lens Course which resulted from this project is provided to assist educators in accredited institutions to impart eyecare and contact lens knowledge. All the contributors deserve recognition for their selflessness and talent.

Debbie Sweeney

President of IACLE

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Educators Guide to the IACLE Contact Lens Course

Overview

The IACLE Contact Lens Course is a comprehensive package of educational materials and other resources for teaching the subject of contact lenses. This package was designed to encompass *The IACLE Contact Lens Course Syllabus* and consists of 360 hours of lectures, practicals and tutorials in ten modules. It contains material at basic, intermediate and advanced levels. The separate document, *The IACLE Contact Lens Course Syllabus,* summarizes the course and includes outlines of Modules 1 to 10.

The teaching resources have been designed for flexibility. They allow the educator to select the materials appropriate to the students' knowledge and the educational requirements of the class, school, institution or country.

The English language reference used for the IACLE Contact Lens Course is: Brown L (Ed.). *The New Shorter Oxford English Dictionary.* 1993 ed. Clarendon Press, Oxford (UK). The only spelling exception is *mold* and *mould.* The Oxford dictionary suggests *mould* in all contexts. We chose to use *mold* for manufacturing-related matters and *mould* for fungi since both meanings and spellings appear regularly in contact lens literature. This differentiation is based on common usage. Where words are 'borrowed' from a language other than English, they are reproduced in their native form where possible.

Where standards have been ratified by the International Organization for Standardization (ISO), or where draft ISO standards are at an advanced stage, their relevant terminology and symbology are used. Système International (SI) units of measure are used wherever possible.

Many major contact lens textbooks from around the world, and some important journal articles, are referenced in the Course, and copyright illustrations are reproduced with permission of the original publishers and/or copyright owners. The References section at the end of each unit details the information sources used throughout.

Teaching Resources - Module 6

Module 6 of the IACLE Contact Lens Course consists of the following materials:

1. **Contact lens manual**

   The contact lens manual, containing:

   - Course overviews
   - Lecture outlines and notes
   - Practical outlines, exercises and notes*
   - Tutorial exercises and notes*

     * Not all units contain all these sections.

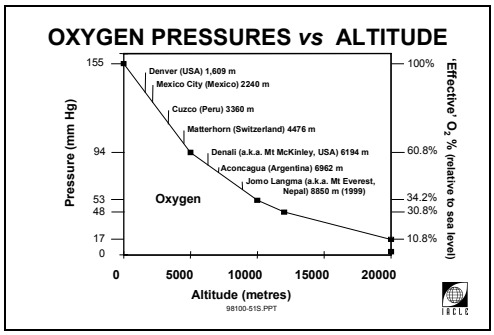
   The recommended allocation of time for the lecture, practical and tutorial components of the module are outlined in the Summary of Module 6 on page *x*. The manual provides recommended activities, references, textbooks and evaluation techniques in the interests of standardization. Ultimately however, the design and methodology of the course is left to the discretion of the contact lens educator.

2. **Slides for lectures, practicals and tutorials**

   The slides have been numbered according to the sequence in which they appear in each lecture, practical and tutorial. Single or dual slide projection can be accommodated. Each slide has an identification code. This code is based on a cataloguing system in use at the IACLE Secretariat and should be used in any communication with IACLE regarding the slides.

For example:

To re-order this slide please quote its identification code



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Symbols, Abbreviations and Acronyms Used in the IACLE Contact Lens Course

SYMBOLS			
↑	increase, high	{	collectively produced by
↓	decrease, low	}	collectively produces
→	produces, towards	↗	sum of
←	produced by, from	±	plus or minus the value of
↔	no change, not obvious	+	plus, add, include, and
↑↑	significant/great increase	–	minus, reduce
↓↓	significant/great decrease	≈	approximately
%	percentage	=	equal to, the same as
<	less than, earlier than	&	and, as well as
>	greater than, after	x°	degrees: e.g. 45°
≥	equal to or greater than	@	in the meridian of
≤	equal to or less than	D	dioptres
?	unknown, questionable	X	axis: e.g. –1.00 X 175. – 1.00D cylinder, axis in 175° meridian
n, n_{sub}, n_{sub}'	refractive indices	Δ	prism dioptres or difference
∝	proportional		

ABBREVIATIONS			
µg	micrograms (.001 mg)	min	minute, minutes
µL	microlitres (.001 mL)	mL	millilitres (.001L)
µm	micrometre (.001 mm) (<1968: micron)	mm	millimetres
µmol	micromoles, micromolar	mmol	millimole, millimolar
cm	centimetres (.01m)	mOsm	milliosmole
d	day, days	nm	nanometres (10^{-9} m)
Endo.	endothelium	Px	patient
Epi.	epithelium	Rx	prescription
h	hour, hours	s	second, seconds
Inf.	inferior	Sup.	superior
kg	kilograms	*t*	thickness
L	litre		

ACRONYMS			
ADP	adenosine diphosphate	LPS	levator palpebrae superioris
ATP	adenosine triphosphate	NADPH	nicotinamide adenine dinucleotide phosphate
ATR	against-the-rule	NIBUT	non-invasive break-up time
BS	best sphere	OD	right eye (Latin: *oculus dexter*)
BUT	break-up time	OO	orbicularis oculi muscle
CCC	central corneal clouding	OS	left eye (Latin: *oculus sinister)*
CCD	charge-coupled device	OU	both eyes (Latin: *oculus uterque* - each eye, or *oculi uterque* - both eyes)
cf.	compared to/with	PD	interpupillary distance
CL	contact lens	PMMA	poly(methyl methacrylate)
Dk	oxygen permeability	R	right
DW	daily wear	R&L	right and left
e.g.	for example (Latin: *exempli gratia*)	RE	right eye
EW	extended wear	RGP	rigid gas permeable
GAG	glycosaminoglycan	SCL	soft contact lens
GPC	giant papillary conjunctivitis	SL	spectacle lens
HCL	hard contact lens	TBUT	tear break-up time
HVID	horizontal visible iris diameter	TCA	tricarboxylic acid
i.e.	that is (Latin: *id est*)	UV	ultraviolet
K	keratometry result	VA	visual acuity
L	left	VVID	vertical visible iris diameter
LE	left eye	WTR	with-the-rule

Summary of Module 6: The Cornea in Contact Lens Wear

Course Program

Lecture			Practical Session			Tutorial (Small Group Teaching)		
Title	***Hrs***	***Level****	***Title***	***Hrs***	***Level****	***Title***	***Hrs***	***Level****
L 6.1 Corneal Oxygen Requirements and the Effects of Hypoxia	1	3						
L 6.2 Corneal Oxygenation with Contact Lenses	1	3						
L 6.3 Contact Lens Characteristics and Oxygen Transmission	1	3						
L 6.4 Microbiology and Contact Lens Wear	2	3						
L 6.5 Ocular Host Defence Systems and Contact Lens Wear	2	3						

* Level 1 = Basic: essential knowledge
Level 2 = Intermediate: desirable knowledge
Level 3 = Advanced: useful knowledge

Course Time Allocation

Level	Lecture	Practical (Laboratory)	Tutorial (Small Group Teaching)	Total Hours
Basic	0	0	0	**0**
Intermediate	0	0	0	**0**
Advanced	7	0	0	**0**
TOTAL	**7**	**0**	**0**	**7**

Request for Feedback

This is the first edition of the IACLE Contact Lens Course, and it is our intention to revise and update it periodically. To ensure each revision is an improvement on its predecessor, we request your help. We invite you to provide feedback in the form of comments, corrections or suggestions which you believe will enhance the accuracy or quality of the Course. Such feedback may then be incorporated in subsequent revisions. We are particularly interested in receiving corrections to, and suggestions for improvements in, the text and slides of the lecture.

To facilitate this feedback process a *pro forma* is included on the next page. This can be photocopied. Please complete your contact details as the team may wish to discuss your suggestions in greater detail or even seek your assistance with any revision resulting from your input.

The IACLE Contact Lens Course
Feedback / Corrections / Suggestions Form

Name: _____ Date: _____
(dd-mm-yy)

Institution: _____

Address: _____

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Module: _____ Unit: _____ Page Number: _____

Slide Code: _____ Section: _____

Comments:

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Response #: _____
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Unit 6.1

(1 Hour)

Lecture 6.1: Corneal Oxygen Requirements and the Effects of Hypoxia

Course Overview

Lecture 6.1: Corneal Oxygen Requirements and the Effects of Hypoxia

I. Oxygen Supply to the Eye

II. Measurement of Corneal Oxygen Demand

III. Equivalent Oxygen Percentage

IV. Effects of Hypoxia

Lecture 6.1

(1 Hour)

Corneal Oxygen Requirements and the Effects of Hypoxia

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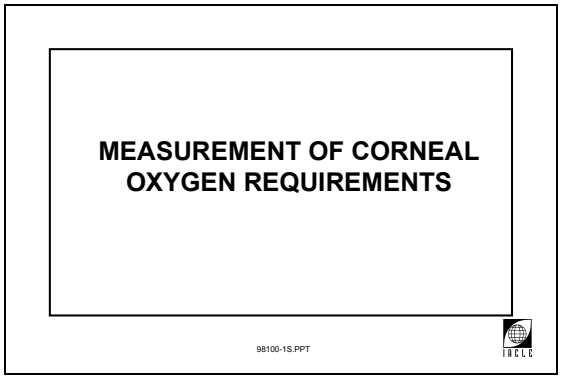
I Oxygen Supply to the Eye

1



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Measurement of Corneal Oxygen Requirements

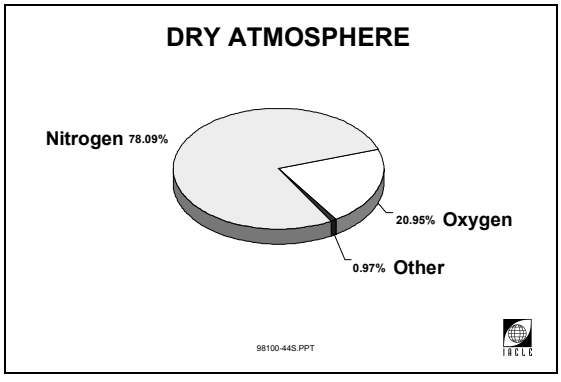
Any reduction in the amount of oxygen available to a metabolically active tissue can significantly alter the physiological equilibrium of the component cells and therefore, the tissue itself. The cornea is no exception.

An adequate supply of oxygen to the cornea is vital to its metabolic processes and the maintenance of its structural integrity. For successful contact lens wear, the lenses fitted must supply at least the minimum level of oxygen the cornea requires. While it would be useful to know the minimum oxygen requirement of the individual cornea, this is usually not practical in routine contact lens practice. Instead, a contact lens is selected which should allow a level of oxygen above, and preferably well above, the 'average' minimum required.

A considerable amount of research has been undertaken to determine the critical oxygen needs of the cornea. While there are some differences in the conclusions of such research, there is general agreement on the oxygen levels required for safe daily and overnight wear of contact lenses. Until recently, many of the contact lenses marketed were incapable of meeting the relatively high levels of oxygen that much of the published research indicated was required, especially in relation to overnight extended wear. Products are now becoming available which exceed the recommendations for this mode of wear by a comfortable margin.

I.A The Atmosphere

2



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Gaseous Components of the Atmosphere

A substantial but minor part of the earth's atmosphere is made up of gaseous oxygen, which is the 'fuel' for many of the metabolic functions within the body. Oxygen makes up approximately 21% of the earth's atmosphere (slide 2).

Other constituents of potential relevance to contact lens wear and/or the cornea are carbon dioxide (CO_2) (slide 3) and water vapour. However, in the normal atmosphere the CO_2 levels are so low as to be practically insignificant and the amount of water vapour present is very variable and will be dealt with separately (see later).

The other components, most of which are the so-called inert gases, have little relevance to contact lens wear. Some of the gases are atmospheric pollutants that may be natural (e.g. methane) or mostly man-made (e.g. nitrous oxide). They may have relevance to the body in general rather than the eyes in particular.

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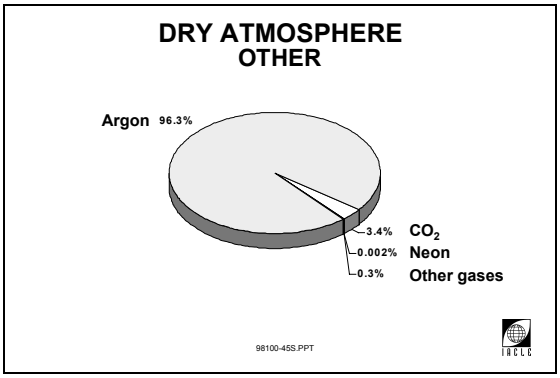

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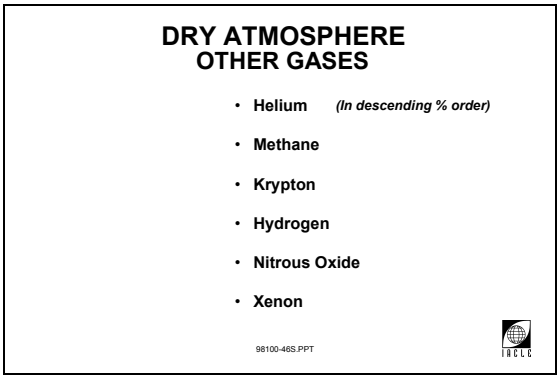

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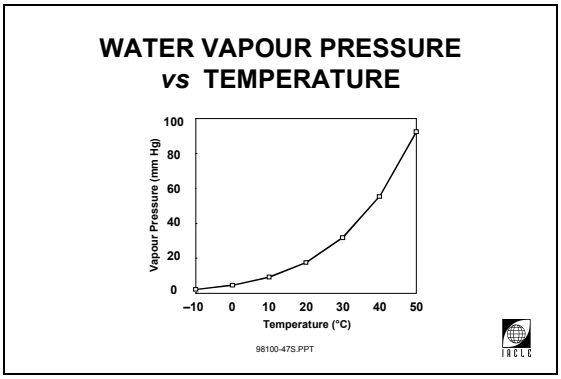

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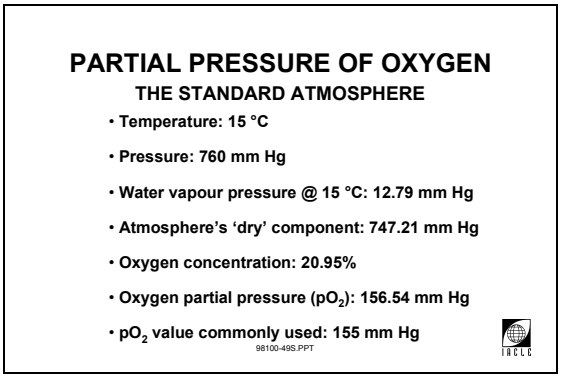

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Partial Pressure (Tension) of Oxygen

In percentage terms, the components of the atmosphere do not vary with altitude. However, the pressure of the atmosphere on the body (atmospheric pressure or barometric pressure) decreases significantly with altitude.

There is also an attendant decrease in the partial pressure of oxygen. However, as a percentage of the total atmospheric pressure, it remains the same, i.e. 20.95% of the total.

At sea level the partial pressure of oxygen in the atmosphere depends on many factors including the barometric pressure, the relative humidity (RH), air temperature and other prevailing factors such as wind, pollution, etc.

Relative humidity (RH) is a significant variable which often depends on local geography, e.g. a sea-side location or a land-locked one, wind direction, e.g. the prevailing winds passing over a body of water or over a hot, dry land mass, and season of the year, e.g. summer or winter.

Importantly, relative humidity changes can affect the water content of conventional hydrogel lens materials. A low relative humidity will decrease the water content of a lens *in situ*. This will result in a decrease in the availability of oxygen under the lens because of the lowered Dk of the material resulting from dehydration.

Somewhat paradoxically, the *standard atmosphere* has 0% RH, an impossibility in reality. The partial

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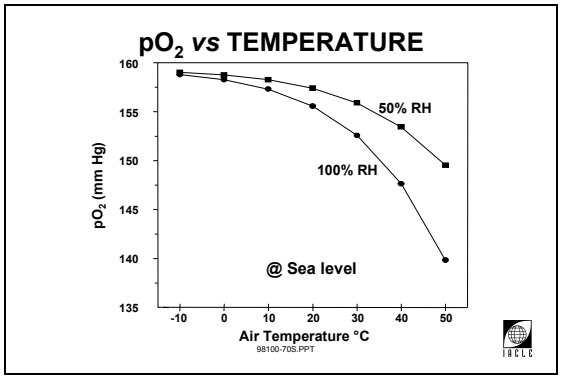

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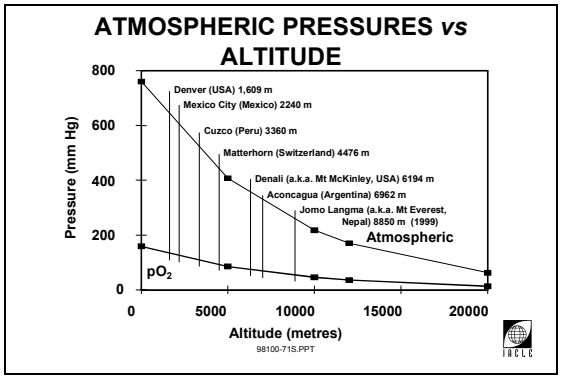

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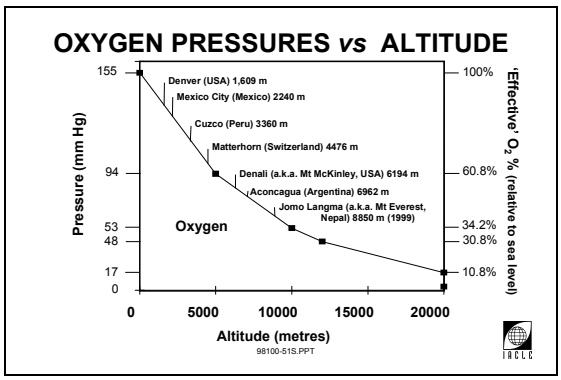

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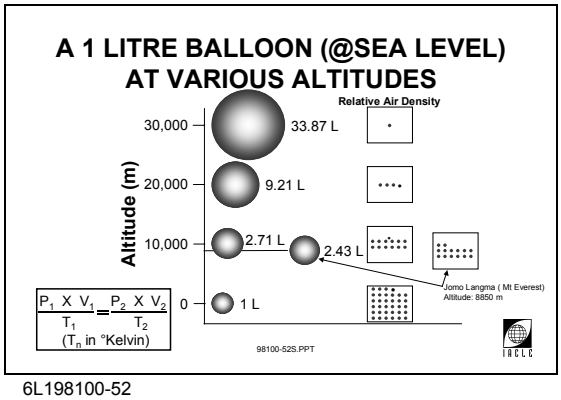

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oxygen pressure in this dry standard atmosphere is 159 mm Hg, i.e. 20.95% of 760 mm Hg. This figure also appears in the literature.

While a standard atmosphere is defined, its component values are somewhat nominal because of the number and efficacy of the variables that can influence them. Although minor, even the latitude at which an atmosphere is measured has some influence.

The components of dry air are subject to less local variation (at sea level) (see pie chart representations in slides 2 and 3). The 'water vapour over water' vapour pressure, i.e. the saturated vapour pressure, varies with temperature (see slide 5). The water vapour pressure over water (12.79 mm Hg) at 15°C, the temperature of the 'standard atmosphere', gives a nominal value which can be used to calculate a pressure of the dry atmosphere (slide 6). From this result an oxygen partial pressure (pO_2) can be derived, i.e. 20.95% of the *dry* total. This is 156.54mm Hg. If the RH is 50%, the 'water vapour pressure over water' value should be halved and the pO_2 recalculated (157.8 mm Hg). This effect is shown graphically in slide 7. Slide 7 appears to exaggerate the effect because of the range chosen for the ***y*** (pO_2) scale.

For simplicity, a value slightly lower than calculated, i.e. 155 rather than 156.54, is used in most published works. The inaccuracy introduced is of the order of 1% which is of little practical consequence in the light of the variability of the actual value under real-world circumstances.

The most influential factor is altitude. The value 155 mm Hg is used as the oxygen partial pressure at sea level in this lecture, as it is the value that has been used for many years.

The effect of altitude on atmospheric pressure, and oxygen partial pressure, is illustrated graphically in slide 8. This theme is expanded in slide 9 which plots the percentage (relative to that at sea level) and partial pressures of oxygen, against the altitude of various geographical features or inhabited areas from around the world.

Using standard equations it is possible to calculate the volume a gas would occupy under various atmospheric conditions. Slide 10 shows the volumes and the apparent size (diameter) a 1 litre balloon (at sea level) would have at altitudes to 30,000 metres. The physical properties and influence of the balloon itself are assumed to be zero and are ignored.

The relative air densities are also shown graphically. Air temperature and altitude are not directly related, i.e. the air temperature does not simply get colder and real data has been used in the underlying calculations. The data for the highest point on earth, Jomo Langma (Mt Everest) is shown for comparison. At Jomo Langma's peak, the atmospheric pressure is about 252 mm Hg of which

51-53 mm Hg is contributed by oxygen, i.e. the 'effective' oxygen availability is approximately one third of the value at sea level. This implies that three times the volume of 'air' would have to be inhaled on top of Jomo Langma as would be inhaled at sea level, to access the same amount of oxygen. The effort required to do so is not sustainable and oxygen masks are usually employed. There are other adverse responses to altitude by the body but these will not be dealt with here.

The cornea depends largely on the oxygen available to it, rather than being able to 'adapt' by invoking an active process to compensate for oxygen deprivation. This applies to the deprivation due to altitude as well as the deprivation due to the wearing of contact lenses.

In flight, commercial aircraft maintain cabin pressures much higher than the pressure outside the fuselage so as not to produce hypoxia and/or breathing difficulties in passengers. This process is called 'pressurization' and the cabin is said to be 'pressurized'. Pressures of between 550 and 600 mm Hg, of which 115-126 mm Hg are due to the oxygen component, are used normally. If the destination is located at high altitude less pressurization is applied to aid acclimatization on arrival (data from a member of the Aviation Medicine E-mail group, 1999).

A few studies have used similar artificial environments to simulate contact lens wear at high altitudes. Strath and Banister (1991) simulated conditions at 4267 metres and found twice the corneal swelling (8% *versus* 4%) and three of seven subjects exhibiting vertical striae. Their results suggested a minimum oxygen level of 12.2% is required to prevent significant corneal changes.

I.B Oxygen Levels in the Eye

11

OXYGEN SUPPLY TO THE EYE

- Atmosphere
- Ophthalmic artery
  - anterior chamber
  - limbus
  - palpebral conjunctiva

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Oxygen Supply to the Eye

Unlike the cornea, the eye as a whole receives most of its oxygen supply via the ophthalmic artery, a branch of the internal carotid artery.

The ophthalmic artery branches into the retinal and ciliary circulatory systems. It is the latter that provides some oxygen to the cornea via the vessels of the anterior chamber, limbus and palpebral conjunctiva. Bulbar conjunctival vessels may also contribute in a small way, since they can also be observed to be dilated at initial eye opening following a period of sleep.

Regardless of the route, or routes, involved in corneal oxygenation, all are indirect with the exception of the limbal region. It is generally assumed that the limbal vasculature provides oxygen for the most peripheral 1 mm of the cornea only (Benjamin, 1994).

Since the cornea proper is avascular, the majority of its oxygen supply is provided by the atmosphere, the variability and properties of which have already been dealt with.

Even the critical atmospheric source of oxygen is indirect since atmospheric oxygen must first dissolve into the tear film before it becomes available for use in the metabolic activities of even the most superficial layers of the cornea. The supply to the deeper layers is even less direct. However, the deeper layers are also supplied by the aqueous humor.

During eye closure, the vasculature of the palpebral conjunctiva plays an important role in anterior eye oxygenation because the lids covering the eye deny the cornea access to the atmosphere. As will be seen later however, the supply system behind closed lids cannot deliver as much oxygen as is received by the open eye.

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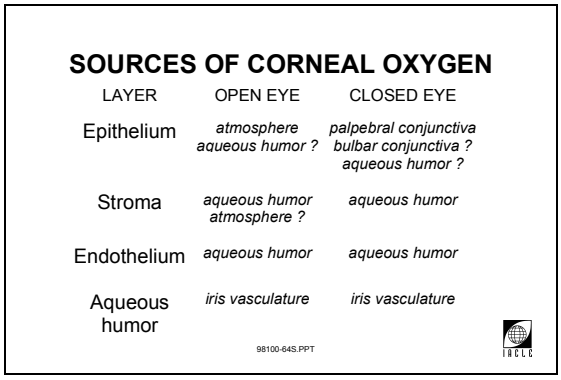

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Sources of Corneal Oxygen

It has been suggested that almost the sole source of oxygen for the epithelium is the atmosphere (in the *open* eye) and the palpebral conjunctival vasculature (in the *closed* eye).

Further, the endothelium is supplied solely by the aqueous humor (Weissman *et al*., 1981). Riley (1969) and Fatt *et al.* (1974) demonstrated a net flux of oxygen from the anterior chamber into the cornea even in the open eye. This means that the endothelium is supplied by the aqueous humor even in the open eye, while in the closed eye it is dependent on this source (Fatt *et al.,* 1974).

Hamano *et al.* (1986b, fig. 4 in article) showed that in the rabbit eye, when a PMMA contact lens is worn, the stromal pO_2 is higher than the anterior corneal pO_2. This suggests the anterior chamber as the source of oxygen, at least as far forward as the anterior stroma. A similar result had already been shown by Steffansson *et al.* (1983) in the cat. Their report showed that when an impermeable contact lens is worn, the oxygen tension in the anterior chamber decreases due to an increased oxygen flux into the cornea.

Zantos' discovery of endothelial blebs in the *in vivo* cornea (see Zantos and Holden, 1977) also demonstrated that the endothelium is not entirely independent of the external eye environment, albeit by mechanisms unknown.

Endothelial blebs are changes in the appearance of the endothelial mosaic observed soon after the introduction of a contact lens, hypoxia/anoxia or experimental gases containing carbon dioxide in the presence of normal amounts of oxygen (see Holden *et al.,* 1985). This 'influence' of the external environment may not be oxygen mediated however. It may be lactate mediated as first suggested by Riley (1969), the result of a pH decrease as suggested by Holden *et al.* (1985), or simply pH changes (Williams, 1986).

However, the endothelium and stromal keratocytes are almost solely dependent on the aqueous humor for oxygen. The other corneal components are not active metabolically. Riley (1969), suggested that in

the rabbit eye at least, about 20% of corneal oxygen was derived from the aqueous humor.

In the past, the source of aqueous humor oxygen was assumed to be the ciliary body and the iris vasculature. More recently however, Hoper e*t al.* (1989) showed that the source was probably just the iris vasculature.

This conclusion was reached after a comprehensive study of the pO_2 values at many places in the anterior chamber adjacent to the iris itself, in rabbits and monkeys. The values in front of the pupil were very low, and were much higher in the periphery. Further support for their claim was provided by a repetition of their measurements following partial and complete iridectomies.

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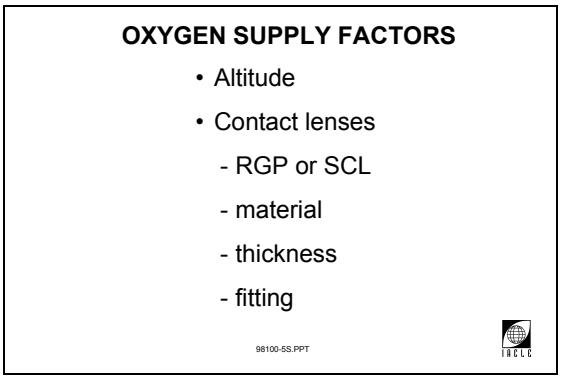

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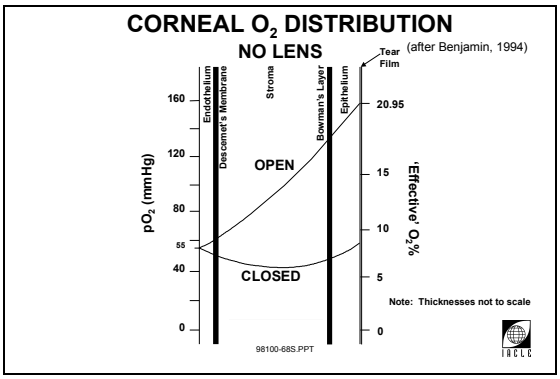

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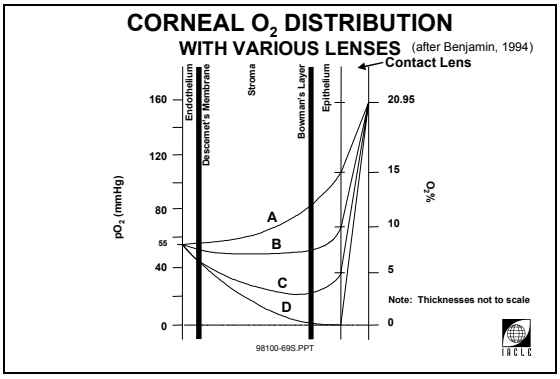

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Determinants of Oxygen Supply to the Cornea

Oxygen tension levels vary across the cornea and within the cornea. The highest level is at the anterior surface. This is perhaps fortunate because the epithelium is the most metabolically active of the cornea's layers and its structural and functional integrity is critical to its barrier function.

The oxygen tensions and percentage distributions across the corneal layers are shown (slide 14) for the following:

- Open eye, central
- Closed eye, central

The oxygen tension at the superior anterior surface of the cornea is reduced by the presence of the eyelids to an extent that varies from individual to individual.

Behind closed eyelids, atmospheric oxygen is unavailable. Under closed eye conditions oxygen to the anterior eye is supplied from the palpebral conjunctival vessels principally, the bulbar conjunctiva and minimal amounts from the limbal vasculature.

Since the driving force of the oxygen supply to the anterior eye is atmospheric pressure, the reduced availability at high altitudes (reduced partial pressure) means the amount of oxygen absorbed is reduced and the level in corneal tissue is lower.

The wearing of contact lenses reduces the supply of oxygen to the cornea. The type of contact lens, i.e. RGP or SCL, as well as lens factors such as material and thickness affect the flow of oxygen to the cornea. All result in a lower oxygen supply to the anterior surface of the cornea.

Slide 15 shows the oxygen tension and percentage distribution across the corneal layers for lenses that allow the following oxygen tensions at the central anterior surface:

- Lens A – 15%
- Lens B – 10%
- Lens C – 5%
- Lens D – 0%

The fitting characteristics of a lens may also play a role, albeit minor, in oxygen supply. Whether rigid or flexible, any lens that permits a greater exchange of tears with each blink will provide more oxygen than a lens that is 'tight' and/or immobile on the cornea.

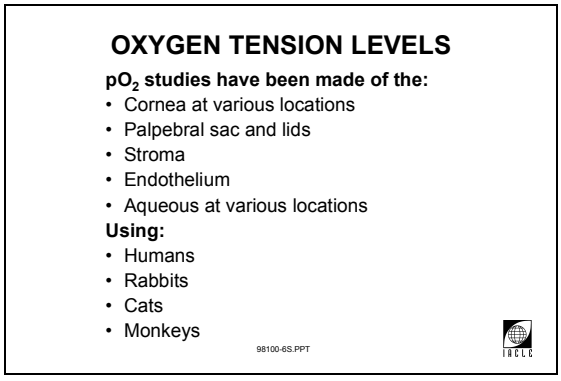
Typically, the contribution of fit to corneal oxygenation is much more significant with RGP lenses. Fatt and Lin (1976) and Decker *et al.* (1978) showed that the 'tear pump' under soft lenses was a minor contributor to corneal oxygenation. Using a fluorophotometer, Polse (1979) showed that when SCLs were worn, each blink only exchanged about 1.1% of the tear volume. On the other hand, RGPs exchange some 10 to 20 times this amount.

Recently, these figures were confirmed in a study by McNamara *et al.* (1999). Their study, again using fluorophotometry, showed exchange rates of between 1.24% and 1.82% per blink when SCLs were worn. Interestingly, smaller SCLs (TD: 12 mm) resulted in greater exchange rates but even these lenses only gave 'modest' results when compared with RGP contact lenses.

However, tear exchange is only a part of a larger scenario. Once other critical factors such as oxygen permeability (Dk), lens thickness, area of cornea covered, etc. are taken into account, RGP lenses deliver approximately three times more oxygen to the cornea than SCLs (Mandell *et al.*, 1987).

Developments since this finding including higher permeability materials, have probably increased the lead RGP materials currently enjoy. The nascent SCL category of siloxane and fluoro-siloxane-containing low-water hydrogels whose Dks can match or exceed those of RGP materials, will reduce, or even eliminate, the margin presently enjoyed by RGP materials despite the latter not covering all of the cornea.

16



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Ocular Oxygen Levels: Open and Closed Eye

The oxygen tensions (partial pressures) at various points in the eyes of several animal species have been measured or estimated. The results are often based on assumptions extrapolated from *in vitro* data and may be method-dependent.

An atmospheric pressure of 760 mm Hg at sea level is assumed. Therefore, in the open eye, the anterior corneal surface is exposed to a partial oxygen pressure (pO_2) of 155mm Hg (derived previously).

Many papers draw attention to the significant individual variation found in the pO_2 levels and/or O_2 consumption rates measured for both animal and human studies, e.g. Mandell and Farrell, 1980, Holden *et al.*, 1984, Lin, 1992. Lin also suggests that the oxygen consumption rate varies over time within an individual.

The published open-eye oxygen tensions at various levels of the anterior eye are summarized below.

17

SUMMARY of pO_2 DATA
OPEN EYE

Location	Human	Rabbit	Cat
Under Lids	33.7 – 61.4	38.9	
Under CLs	0 – 82.3	0.58 – 112	
Anterior Chamber	50 – 59.7	7.7 – 65	30 - 37
Ant. Chamber with CLs	25 - 75	6 – 27.73	13

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18

SUMMARY of pO_2 DATA
CLOSED EYE

Location	Human	Rabbit
Under Lids	50 - 67	
Under CLs	0 - 35	
Anterior Chamber	55	8.7
Anterior Chamber with CLs		5.1 – 12.9

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Summary of Published pO_2 Data

All Species:

- At the anterior ocular surface:
  - 151 to 159 mm Hg

(Benjamin and Hill, 1988, Benjamin, 1994, differences partly due to laboratory altitudes).

Open eye, HUMAN:

- Under the lids:
  - 33.7 to 61.4 mm Hg
- Under contact lenses:
  - 0 to 82.3 mm Hg
- In the anterior chamber:
  - 40 to 59.7 mm Hg
- In the anterior chamber , contact lenses on:
  - 25 to 75 mm Hg

(Benjamin, 1994, Hamano *et al.*, 1986, Serdahl *et al.*, 1989, Efron and Carney, 1979, Holden and Sweeney, 1985, Fatt *et al.*, 1974, Weissman, 1986, Polse and Decker, 1979, Rasson and Fatt, 1982, Hamano, 1985, O'Neal *et al.*, 1983, Friedenwald and Pierce, 1937, Kleifield and Neumann, 1959, Fatt and Bieber, 1968, Fatt, 1978, Ruben, 1975, Benjamin, 1994, Thiel, 1967, Fatt and Ruben, 1993)

Open eye, RABBIT:

- In the conjunctival sac:
  - 38.9 mm Hg
- Under contact lenses:
  - 0.58 to 112 mm Hg
- In the anterior chamber :
  - 7.7 to 65 mm Hg
- In the anterior chamber, contact lenses on:
  - 6 to 27.73 mm Hg

(Hamano *et al.*, 1986, Fatt and Lin, 1985, Hamano, 1985, Ichijima *et al.*, 1998, Harvitt and Bonanno, 1996, Kwok, 1985, Kleinstein *et al.*, 1981, Stefansson *et al.*, 1987, Barr and Roetman, 1974, Barr and Silver, 1973, Hoper *et al.*, 1989, Kleinstein *et al.,* 1981, Stefansson *et al.,* 1983, Mc Laren *et al.*, 1998)

Open eye, CAT:

- In the anterior chamber :
  - 30 to 37 mm Hg
- In the anterior chamber with PMMA contact lenses on:
  - 13 mm Hg

(Fatt *et al.*, 1982, Kwok, 1985, Stefansson *et al.*, 1983)

Open eye, monkey:

- pO_2 anterior chamber :
  - 41.5 to 13.9 mm Hg depending on sensor position

(Hoper *et al.*, 1989)

Closed-eye, HUMAN:

- At the central cornea:
  - 50 to 67 mm Hg
- In the anterior chamber:
  - 55 mm Hg (Ruben, 1975).
- Under contact lenses:
  - 0 to 35 mm Hg

(Kwan and Fatt, 1970, Benjamin, 1982, Fatt and Bieber, 1968, Fatt and Lin, 1985, Fatt, 1987, Benjamin, 1994, Ruben, 1975, O'Neal *et al.*, 1983, Ichijima *et al.*, 1998)

Closed-eye, RABBIT:

- In the anterior chamber:
  - 8.7 mm Hg
- In the anterior chamber, RGP lenses on:
  - 5.1 to 12.9 mm Hg

(Barr and Silver, 1973, Hamano *et al.*, 1986)

Anterior anoxia, human:

- Under lid:
  - 42.8 mm Hg

(Efron and Carney, 1979)

Anterior anoxia, rabbit:

- pO_2 anterior chamber:
  - 9.6 ±2.9 mm Hg

(Barr and Silver, 1973).

II Measurement of Corneal Oxygen Demand

19

MEASUREMENT TECHNIQUES

Invasive:

- Polarographic oxygen sensor (O_2 uptake)
- Glycogen reserves
- Cell mitosis
- Loss of sensitivity (aesthesiometry)

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20

MEASUREMENT TECHNIQUES

Non-invasive:

- Corneal swelling (pachometry, goggles)
- Corneal changes (slit-lamp biomicroscopy)
- Redox fluorometry

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Measurement of Corneal Oxygen Demand

There are a number of techniques available to measure the cornea's demand for oxygen and assess the effects of hypoxia on the structure and function of the cornea.

Some of these techniques, e.g. measuring the rate of epithelial cell mitosis, are only available as research laboratory tools. The techniques used can also be categorized as invasive or non-invasive. The former is defined as any technique that touches the eye or requires a biopsy specimen of the eye to be 'harvested'. The latter refers to any method which takes a 'look but no touch' approach.

Clinically, the slit-lamp biomicroscope provides the contact lens practitioner with a simple, non-invasive means of observing the effects of reduced oxygen supply to the eye *in situ.*

Frequently, clinical techniques are qualitative rather than quantitative, i.e. descriptions rather than numbers or ratings are used to 'describe' an observation. This is usually because such techniques are simpler, quicker and easier to perform, less expensive and often easier to understand.

21

OXYGEN CONSUMPTION

- Measurement of oxygen flux across the tear - epithelial interface
- Micro-polarographic electrode
  - oxygen sensitive
  - membrane cover (polyethylene)
  - measures epithelial O_2 consumption

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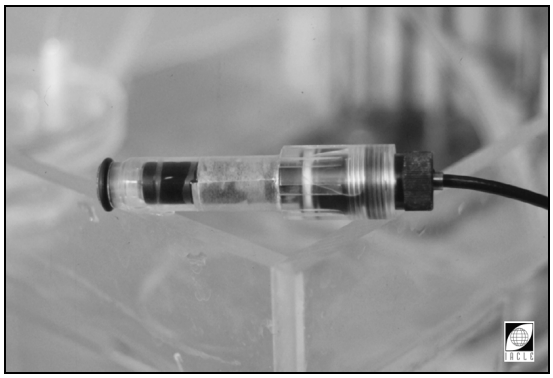
Measurement of Corneal Oxygen Consumption

Normal metabolic activity of the cornea is dependent on an adequate supply of oxygen from the atmosphere. The oxygen flux into the cornea is driven by the needs of the cornea's basal metabolic rate. A steady state is maintained as long as there is no alteration in oxygen supply or availability.

Measurement of the rate at which oxygen is taken up by the cornea is usually made with a small Clarke-type polarographic oxygen sensor (Clarke, 1952-1956, see the Foreword in Fatt, 1976). This type of sensor was first used on the cornea by Hill and Fatt (1963).

II.A The Polarographic Oxygen Sensor

22



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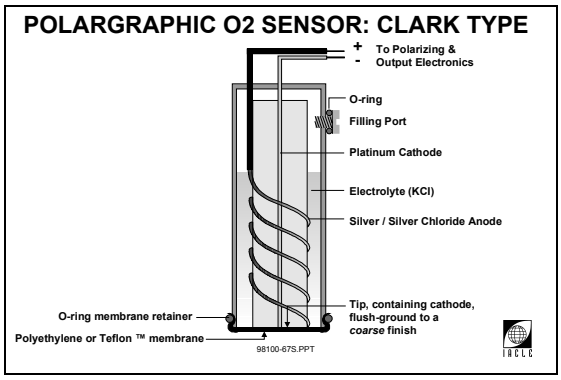

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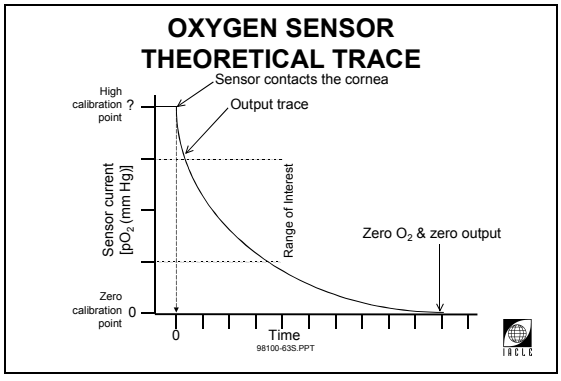

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Clark-type Polarographic Oxygen Sensors

This is a liquid-filled (electrolyte), membrane-covered, bipolar, polarographic electrode developed by Clark *circa* 1952. Basically, the two electrodes (the cathode and the reference anode, hence the term bipolar) required are mounted side-by-side and the combination is isolated from the liquid or gas to be measured by an oxygen permeable membrane. The latter behaves as a barrier to all other significant chemical species (after Fatt, 1976). The original membrane used was polyethylene but other polymers have been used. Alternative materials include Cellophane™, Teflon™, dialysis tubing, polypropylene and siloxane rubber.

Commonly, the electrode materials are platinum (cathode, negative) and silver or silver/silver chloride (anode, positive). However, other noble metals such as gold, rhodium and silver have also been employed, the latter used in conjunction with a lead anode.

Slide 22 shows a Radiometer E5047 oxygen sensor.

Commonly, the electrolyte is potassium chloride (KCl) used in a concentration of between 0.1 and 0.5 Molar. Potassium hydroxide (KOH) and borate buffer solutions have also been used. If the electrolyte is not borate based, it may be buffered to help solution stability.
(*Note:* A one molar (1M) solution is a one litre (1L) solution, containing one mole [1 gram molecular weight] of solute.)

Electrode separation has been achieved with glass (most commonly) and some epoxy resins. Other epoxy resins are unsuitable because they can hydrate and provide an alternative conduction path. To achieve electrolyte 'bridging' between electrodes when a membrane is stretched tightly over the face of the combination of electrodes, either their surface is ground roughly or a layer of rice paper, filter paper or similar porous 'spacer' is used.

The key characteristic of a polarographic oxygen electrode is the essentially linear relationship between electrode current and the partial pressure of oxygen in the fluid being measured. Unfortunately, most real electrodes are not truly linear over their total range of operation and all need to be calibrated at two points at least, within the desired range of measurements. Only in this way can reasonably accurate oxygen partial pressures be obtained.

A theoretical trace of a polarographic oxygen sensor is shown in slide 24.

25

POLAROGRAPHIC OXYGEN SENSORS PERIPHERALS

- Polarizing unit
- Output device (or interface)
  - integral display (analogue or digital)
  - output to external data logger:
    - chart recorder
    - computer

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Polarographic Oxygen Sensors: Peripherals

In addition to the oxygen electrode itself, an external polarizing unit (a source of electrical energy) and an output device or interface are also required.

The polarizer provides a constant voltage of between 0.5 to 1.0 volts to the electrode, although most operate between 0.6 and 0.8 V. Superior results are obtained if the polarizer is an auto-sensing device that maintains a constant applied voltage over a wide range of measuring circumstances.

Output devices translate an electrode's current into a form of greater utility. Such a form may be:

- An amplified (larger) current
- A voltage
- A direct readout of oxygen tension (once suitably calibrated).

26

POLAROGRAPHIC OXYGEN SENSORS BASIC REQUIREMENTS

- Reproducible current for a given pO_2
- Rapid settling time when pO_2 is stable
- Rapid response to changes in pO_2
- Constant current for constant pO_2

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Polarographic Oxygen Sensors: Basic Requirements

27

POLAROGRAPHIC OXYGEN SENSORS CHEMISTRY e = electron

Cathode (– ve, Pt or Au):

$4\,e^- + O_2 + 2H_2O \rightarrow 4OH^-$

Anode (+ ve, Ag/AgCl):

$4\,Ag \rightarrow 4Ag^+ + 4e^-$

Overview:

$4\,Ag + O_2 + 2H_2O \rightarrow 4Ag^+ + 4OH^-$

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Polarographic Oxygen Sensors: Chemistry

With a polarizing voltage applied to an oxygen sensor, any oxygen present in the electrolyte or entering the electrolyte from the external environment via the sensor's membrane will, in the presence of water, be converted to hydroxyl ions (OH^-) at the cathode (–ve). Essentially, the four electrons required for this conversion of two water molecules into four hydroxyl ions, are provided by a related reaction occurring at the anode (+ve). The silver anode is ionized into silver ions (Ag^+) with the release of electrons (after Hamano *et al.*, 1985 and Fatt, 1992).

To maintain the electrochemical balance, an equal number of electrons are involved at each electrode, i.e. the anode supplies electrons at the same rate as the cathode uses them.

The greater the quantity of oxygen 'available' the greater the conversion rate of oxygen into hydroxyl ions (in the presence of water), and the greater the transfer of electrons from the anode to the cathode. Conventionally, this 'transfer' of electrons is termed a 'current' and in oxygen sensors it is proportional to the oxygen 'availability'. Therefore, an oxygen sensor transduces oxygen availability immediately behind its membrane into a measurable current.

Several mutually exclusive factors in sensor design need to be considered. The magnitude of the current is directly related to the surface area of the electrodes. However, large electrodes 'consume' or 'convert' large volumes of oxygen and deplete or alter the electrolyte rapidly. These internal issues can affect the apparent levels of oxygen available in the fluid being measured, because consumption may exceed the rate the membrane can deliver oxygen to the electrodes.

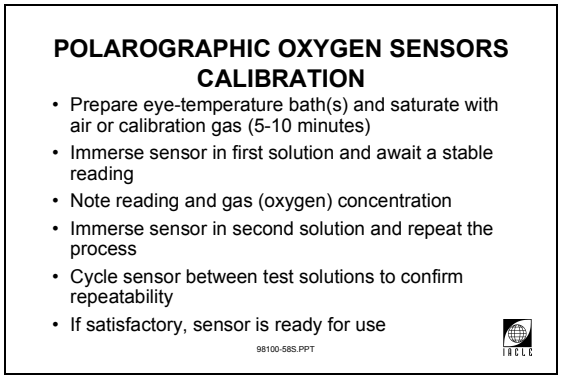
On the other hand, while small or very small electrodes may solve the internal oxygen consumption issue, the smaller currents that result produce their own difficulties. These include poor signal-to-noise ratios and more sophisticated output devices being required. Small sensors are also more susceptible to temperature changes. While some form of temperature sensing can be incorporated into larger sensors to allow automatic compensation, it is more difficult to incorporate into smaller devices.

Another electrode issue is membrane thickness. As with contact lenses, thicker membranes have lower gas transmissibility. Another factor to be covered later is the role the sensor's membrane plays as a gas (oxygen) reservoir, albeit one of limited volume. Thin membranes contain smaller reserves of oxygen, although the solubility of oxygen in the membrane also influences the volume of oxygen stored.

Membrane factors are therefore significant determinants of the speed of response of the sensor overall, and the volume of oxygen contained in such a reservoir plays an important role in corneal oxygen consumption determinations.

Common membranes are 12.5-13 µm thick polyethylene, 12 –14 µm thick Teflon ™ and 12.5 or 27µm polypropylene (after Quinn, 1981).

28



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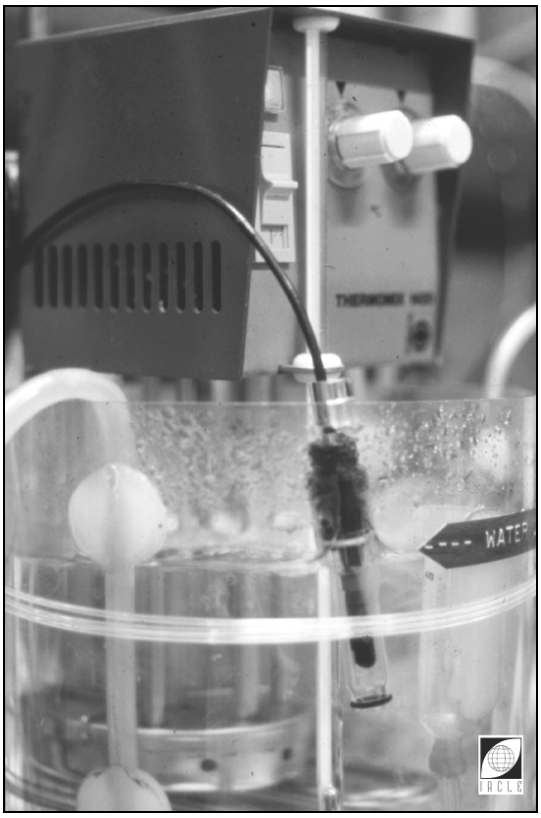
Polarographic Oxygen Sensors: Calibration

Basically, oxygen sensor calibration is usually a two-point process with the zero point being either nitrogen-saturated normal saline or a special-purpose, single-use, zero-oxygen solution. Such solutions are based on sodium hydrosulphite (Fatt, 1978) or sodium sulphite and sodium tetraborate (Ödman *et al.*, 1985).

The high point is chosen to reflect the likely upper value of any determination made. The most common upper value for oxygen is 155 mm Hg, achieved using normal saline saturated with air. Other special calibrated gases with lower oxygen concentrations (the balance is usually food-grade or better nitrogen) can be employed for saturation if a narrower range of values is anticipated. The closer the calibration range is to the actual range measured, the more accurate the results.

29



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Saturation of the calibration solutions is achieved by the vigorous bubbling of the calibration gas through the solution for some time, i.e. 10 to 30 minutes or more depending on the volume of the calibration solution. The latter is maintained at approximately normal eye temperature so that thermal effects, induced by temperature differences, can be avoided.

Normally, a new membrane is fitted for each series of measurements that may take days, or for each measuring session, whichever is dictated by experience and sensor behaviour.

For calibration, the sensor is lowered into the test solution to the extent of the exposed length of the anode (often a relatively short length). Once the output stabilizes, it is recorded against the known oxygen concentration.

In the case of special zero-oxygen solutions, which are usually delivered in break-off sealed glass vials, steps need to be taken to raise the solution temperature uniformly to that of the other calibration solution. This is achieved by immersing the unopened vial in the other calibration solution and agitating it.

Once the electrode is immersed in the zero-oxygen solution for calibration of the zero point, only gentle stirring is appropriate to prevent air being stirred into the solution and a false result being recorded.

Eye temperature is a subject of some conjecture. Most reports place the mean value between 34 and 36°C. In a series of studies by Bruce (1991), the values ranged from 33.8 – 36.6°C. A value for the saline bath should be selected within this range and controlled to within ±1°C during all procedures. Further details on corneal temperature appear in Lecture 1.1, section II.A.5.

Periodic checking/re-calibration during the measuring session is also prudent.

Observations made during use should indicate whether there is a need to change the electrolyte and/or clean and recondition the electrodes of the sensor.

If the sensor's response is too slow, subsequent readings may not be valid. If necessary, the sensor may need a new membrane, a change of electrolyte or an electrode cleaning and/or reconditioning, according to the manufacturer's instructions.

II.B Measuring Oxygen Consumption

30

CORNEAL OXYGEN CONSUMPTION TECHNIQUE

- Membrane saturated with air
- Cornea is applanated by sensor
- O_2 diffuses into anterior cornea
- Rate of depletion of O_2 from membrane estimated

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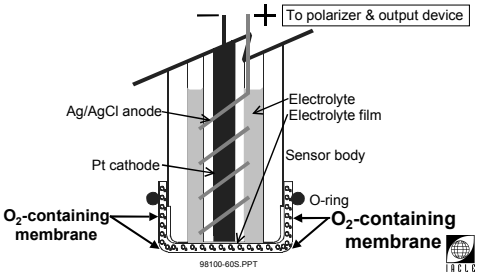
31

OXYGEN SENSOR: MEMBRANE RESERVOIR



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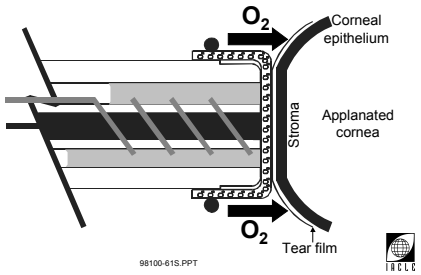
32

OXYGEN SENSOR AT APPLANATION



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Corneal Oxygen Consumption: Technique

The most common technique used to measure the corneal oxygen uptake rate is that pioneered by Hill and Fatt (1964). This method involves a calibrated oxygen sensor, whose membrane reservoir has been charged with normal atmospheric levels of oxygen (slide 31 and 32), being used to applanate the bare cornea (slides 33 and 34).

The oxygen from the membrane reservoir diffuses into the epithelial cells at a rate dictated largely by the needs of the metabolic processes within the corneal epithelium (see the discussion of the possibilities in Hill and Fatt, 1963).

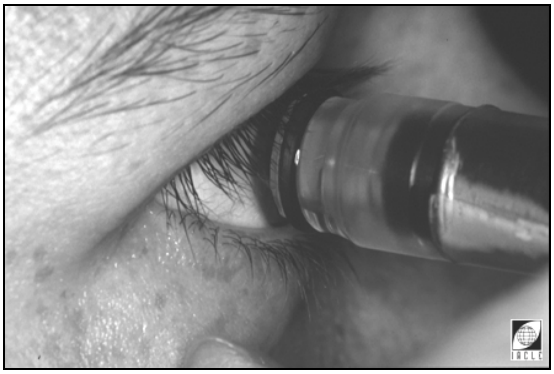
The presence of the probe over the cornea restricts the latter's access to the atmospheric oxygen it needs, a fact confirmed by the rapid depletion of oxygen from the membrane. This depletion is measured as a rapid decline in oxygen sensor current, indicating low levels of oxygen availability inside the sensor after a relatively short period of time (slide 35).

Oxygen charging is done by equilibrating the sensor in a bath of air-saturated normal saline maintained at eye temperature (slide 29). This loads the membrane reservoir with the equivalent of a pO_2 of 155 mm Hg.

Often, physical factors and temperature and applanation/diffusion factors cause the initial part of the recording to be unreliable. To counter this, an analysis of the recording is delayed until the recorded oxygen tension reaches 140 mm Hg and is terminated when the tension is 40 mm Hg for example. A 100 mm Hg pO_2 recording range is therefore used.

An extrapolation of the data to time zero can be used to estimate the physiological circumstances at the commencement of the recording of data. This process is simplified by using computer-based regression analysis.

34



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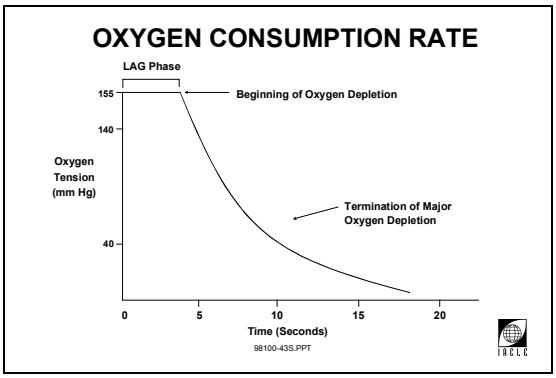

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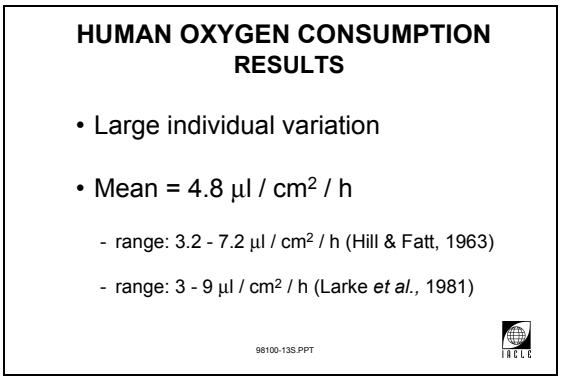

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Human Corneal Oxygen Consumption

The results of extensive research indicate that significant variation exists between individuals (Benjamin and Hill, 1988, Mandell, 1988, Lin, 1992) and within individuals (Lin, 1992).

While it is possible that some of the variation is due to differences in experimental methodology, in many aspects of physiology humans show significant individual variation. Corneal oxygen consumption is probably one of these aspects.

These differences between individuals should be borne in mind when fitting contact lenses and assessing their clinical performance.

38

GOGGLE EXPERIMENTS

- Controlled pre-ocular environment
  - oxygen level
  - temperature
  - humidity
- Range of oxygen concentrations
- Measurement of corneal swelling
  - pachometry

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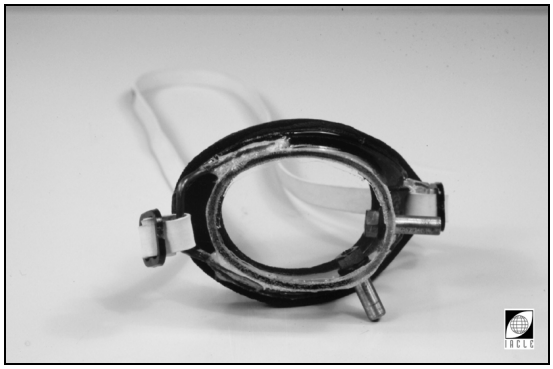
39



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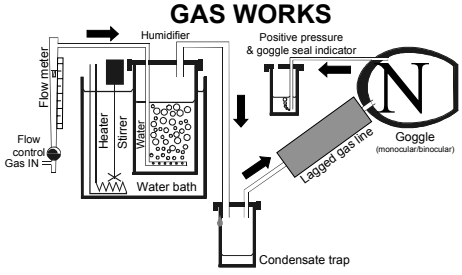
GOGGLE EXPERIMENTS: GAS WORKS



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Experiments with Goggles

Changes in the pre-ocular environment may affect the normal structure and function of the cornea. These effects can be measured or monitored with instruments such as pachometers, fluorophotometers and aesthesiometers. These instruments are discussed in Lecture 9.1.

The use of goggles makes it possible to alter the environment in front of the eye and to examine the effects of such alterations on the cornea.

Typically, a tight-fitting, sealed goggle is used (slide 39) and humidified gases at fixed temperatures are delivered as an artificial pre-ocular environment (slides 40 and 41).

Hypoxia

If the cornea is rendered hypoxic, e.g. by contact lens wear or by decreasing the O_2 concentration inside a goggle, the cornea's oxygen demand increases. This increase is due to an accumulated oxygen debt resulting from a hypoxia-induced depletion of the cornea's aerobic resource glycogen. This occurs as the cornea is forced into the less efficient anaerobic respiration mode.

If an oxygen-equilibrated sensor is applied to the hypoxic cornea, the oxygen flux from the membrane reservoir into the cornea (via the tear film) will be greater than under normoxic circumstances. The greater the debt, the greater is the flux from the sensor into the eye, i.e. they are directly related (Hill, 1994).

As sensor current is the analogue of oxygen availability within the sensor, a rapid decline in sensor current is indicative of a rapid depletion of oxygen from the membrane reservoir. Therefore, it is possible to indirectly measure the state of corneal oxygen deprivation by applying a calibrated oxygen sensor to the cornea immediately after exposure to hypoxic gases or contact lens wear, and measuring the rate of decline of sensor current.

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42

CORNEAL PACHOMETRY

- Sophisticated systems can measure:
  - epithelial thickness
  - stromal thickness
  - total thickness
- Optical pachometry is non-invasive
- Ultrasonic pachometry is more common in anterior eye surgery, but touch required

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Measurement of Corneal Thickness by Pachometry

Changes in the thickness of the cornea can be measured optically with reasonable accuracy using an optical pachometer. In conjunction with goggles, the optical pachometer can also be used to measure the effects of alterations to the pre-ocular environment on corneal thickness.

A number of different types of systems are available (see Lecture 9.1). The optical pachometer is the type most commonly used in clinical research. Being an optical system, its principal advantage is that it is non-invasive. Its main alternative is the ultrasonic pachometer. However, this category of instrument is invasive because corneal touch is required. Ultrasonic pachometers are the type most commonly used in corneal refractive surgery.

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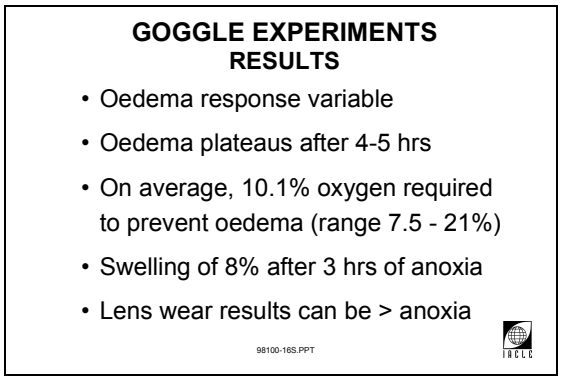

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Results of Goggle Experiments

Studies using pachometry to measure the amount of corneal oedema caused by a reduction in the oxygen supply to the ocular surface indicate that, like oxygen consumption rates, substantial inter-subject variability exists.

A key experiment by Holden *et al.* (1984) indicated that a steady state of corneal swelling is reached after 4-5 hours of exposure to different levels of hypoxia. For their subjects, an average of 10.1% oxygen was required to prevent corneal oedema.

When the pre-corneal environment was made anoxic (100% humidified nitrogen), the level of swelling was found to be 8% after 3 hours.

Interestingly, when a soft lens was worn in anoxic conditions, a higher level of corneal swelling was measured. This indicates that relative hypoxia is not the only factor contributing to corneal oedema with soft contact lens wear (Sweeney, 1991).

III Equivalent Oxygen Percentage

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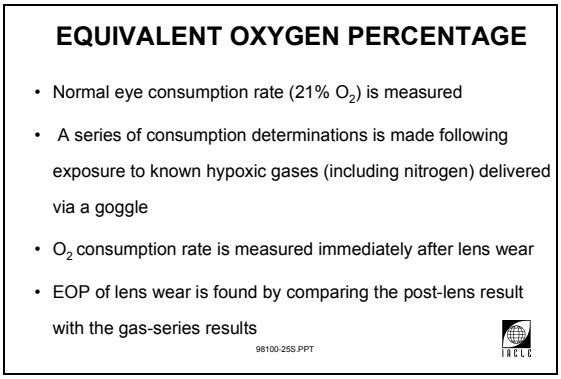

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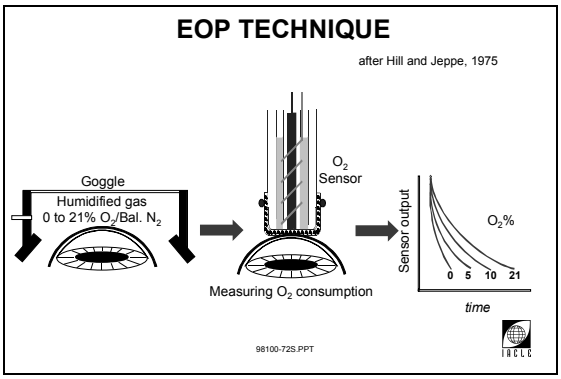

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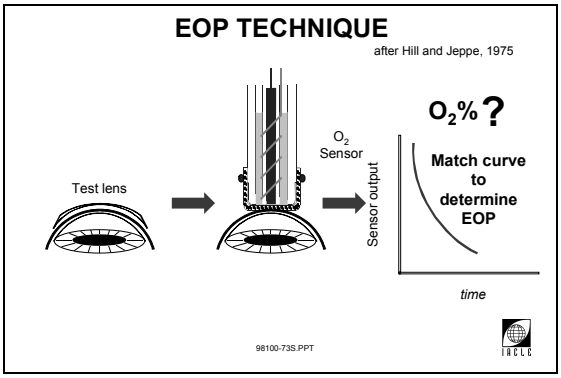

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Equivalent Oxygen Percentage

The equivalent oxygen percentage (EOP) technique indirectly quantifies the corneal environment under a contact lens by ascertaining what oxygen concentration, in an hypoxic gas delivered to the cornea via a goggle, produces an identical corneal response to that produced by the lens.

In the EOP technique, the analogue of 'corneal response' is the cornea's oxygen consumption. This is ascertained from the decline in oxygen sensor output that follows the applanation of the cornea by the sensor.

When the consumption rate (curve) recorded immediately after contact lens wear matches the curve generated immediately after corneal exposure to an hypoxic gas, the equivalent O_2 percentage (EOP) under the lens is said to be the O_2 level present in the matching hypoxic gas. This is because the cornea has an 'equivalent oxygen response' (after Hill and Jeppe, 1975.

The method for measuring the EOP was developed by Hill and Fatt (1963), and utilizes a Clarke-type polarographic oxygen sensor to measure the corneal oxygen consumption rate.

Whereas Dk and Dk/*t* are *in vitro* or laboratory methods of assessing a lens material's oxygen performance, the EOP technique is strictly an *in vivo* or clinical method of assessing a related performance indicator.

The EOP method employed is as follows:

- First, the corneal oxygen consumption rate for the open eye is determined under normal atmospheric conditions, i.e. normoxia.
- A contact lens is then worn for a fixed period of time, i.e. corneal hypoxia is induced by lens wear.
- Immediately after the lens is removed, the corneal oxygen uptake rate is measured (slide 46).
- A series of hypoxia studies, using known gases delivered via a goggle, is then performed on the same eye and its O_2 consumption rates are measured immediately after each gas. Once the consumption curves for each of the known gases have been established (slide 45), they can be used for a comparison with the post-lens wear data.

There are two basic types of EOP determinations; static and dynamic. Static determinations involve 'steady state' conditions, i.e. the results of lens wear *without* the tear pump benefits resulting from any blinking. This form of measuring EOP bears some relationship to the results predicted by calculations of the Dk/*t* of a lens. However, as Hill (1988) points out, EOP is not a Dk or a Dk/*t* value.

The dynamic EOP can not be compared directly with Dk/*t* results, as the latter cannot take the effects of tear pumping into account. Differences between dynamic and static EOP results should be greatest with RGP contact lenses because the tear pumping with RGP lenses has been shown to be significantly greater (after Hill, 1994).

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EQUIVALENT OXYGEN PERCENTAGE
ADVANTAGES

- *In vivo* measurement
- Determined by 'oxygen thirst'
- Normal lens wearing environment
- SCL *vs* RGP lens comparisons possible

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EQUIVALENT OXYGEN PERCENTAGE
ADVANTAGES

- Static *vs* dynamic values
- Correlation between contact lens transmissibility (Dk/*t*) and EOP
- Can derive an EOP curve for a material

98100-27S.PPT

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Advantages of Measuring the Equivalent Oxygen Percentage

The proponents of the EOP technique claim a number of advantages over other methods for determining the level of oxygen beneath a contact lens. The claimed advantages include:

- It is an *in vivo* measurement made on living tissue and therefore provides a more accurate indication of corneal function.
- The results of the corneal oxygen consumption measurement provide an accurate indication of the oxygen need, or thirst, of the cornea.
- Lenses are worn in a normal environment so the effect on the cornea is solely lens-related.
- Any type of lens can be fitted and an assessment made of the effect on the corneal oxygen consumption rate. This permits a direct comparison of SCL and RGP lenses.

Further advantages include:

- In studies, it is possible to control the blink rate during lens wear. This provides an opportunity to examine the effect of blinking on the supply of oxygen under a lens, e.g. static *versus* dynamic conditions or frequent *versus* less frequent blinking.
- The dynamic state is very significant for RGP lenses due to the tear exchange driven by each blink. Blinking should have less effect on a SCL. A high correlation is claimed between the measured Dk/*t* for a given lens and its EOP.
- For any material it is possible to derive an EOP curve by performing the test with lenses of different thickness. Such a curve allows the clinician to select a lens thickness that will provide adequate oxygen supply to the cornea under different wearing conditions such as occurs in extended or continuous wear.

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EQUIVALENT OXYGEN PERCENTAGE
DISADVANTAGES

- O_2 consumption rate affected by more than just contact lens wear
- Potential for error
- Invalid assumptions
- Relationship to other measuring techniques may not be direct

98100-28S.PPT

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Disadvantages of Measuring the Equivalent Oxygen Percentage

A number of potential disadvantages of the EOP technique need to be considered when interpreting the data for a variety of lenses and wearing conditions. These include:

- The rate at which oxygen is used by the cornea may be affected by more than just the transmissibility of the contact lens. Other factors such as osmolality changes, temperature and aplantaion forces may affect the measurements.
- Methodological errors can affect the results for the EOP measurement. These may occur at any point during the process and can involve the sensor, contact lens utilization and the recording system.
- A number of assumptions are made which may be invalid. These include:
  - a steady state of oxygen flow is achieved
  - the time lag to apply sensor to the cornea is insignificant
  - a single measurement is sufficient.
- A direct relationship may not exist between the EOP technique and other measurement systems used to evaluate the oxygen transmissibility of a contact lens. The validity of any such comparisons must be evaluated in each case.
- Variations within individuals over time are insignificant.

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EOP *vs* Dk/*t*

EOP = 1.56 X 10^8 (Dk/*t*) +1.89
Efron & carney, 1981

EOP = 2.06 X 10^8 (Dk/*t*) – 0.07
Fatt & Chaston, 1982

Open eye: EOP = 7.2 X 10^8 (Dk/*t*) – 0.50
Closed eye: EOP = 1.4 X 10^8 (Dk/*t*) – 0.27
Roscoe, 1984

EOP = 6.915 X ln (Dk/*t* *X* 10^9) – 9.778
Holden and Mertz, 1984)

98100-62S.PPT

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EOP *versus* Dk/*t* : **Comparisons**

Given that there are two main ways of studying the oxygen performance of contact lenses, i.e. Dk/*t* and EOP, it is logical that comparisons have been made between the two with a view to deriving relationships between them. Using such relationships it is possible, theoretically at least, to predict one value from the other.

The success of these attempts cannot be judged by the seemingly large differences in some of the equations derived (see slide). In fact there is a 'highly significant' (Efron and Carney, 1981) or a 'strong' correlation (Holden and Mertz, 1984) between the two. The differences are due to the use of different: animal species, lens types, Dks of materials (high only, low only or a mixture), controls over blinking and subject populations (see Fatt and Chaston, 1982, Efron and Carney, 1982).

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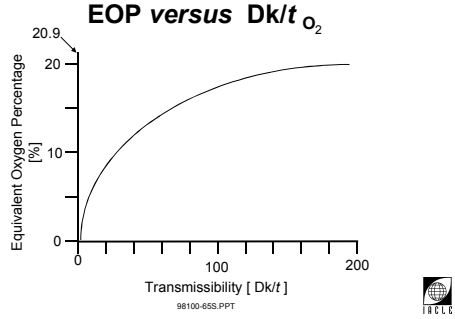

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The graph of EOP *versus* Dk/*t* in slide 51 is based on the work of Hill in general and Holden and Mertz (1984), Hill (1988) and Benjamin (1994).

When powered contact lenses are used, the Dk/*t* varies according to the local thickness whereas the EOP technique encompasses ('averages') some of the effects of regional lens thicknesses. Caution is required when extrapolating from lens centre thickness data (see Fatt *et al.*, 1993, Fatt and Ruben, 1994).

III.A Contact Lens-Induced Corneal Swelling

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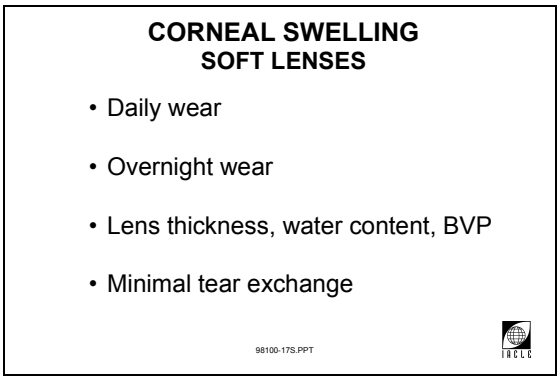

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Corneal Swelling with Soft Contact Lenses

Currently, all conventional soft contact lenses act as physical barriers to the supply of oxygen from the atmosphere and reduce the level of oxygen available to the cornea.

Generally, with conventional SCLs, the higher the water content the greater the oxygen permeability of the material. For a given water content, the thinner the lens, the greater the transmission of oxygen to the cornea.

However, there is only minimal exchange of tears behind an SCL with each blink. This exchange therefore contributes a negligible proportion of the oxygen supplied to the cornea during SCL wear.

With the new generation of highly oxygen permeable SCLs, i.e. those containing siloxanes or fluoro-siloxanes, the oxygen transmissibility performance issue appears to have been solved. However, the supply of oxygen via tear exchange is probably not significantly different from that with earlier lenses.

The effects of hypoxia on the cornea with SCL wear are usually greater when the lenses are worn for longer periods of time. In daily lens wear (DW) a patient who uses their lenses for 7 days a week, 16 hours per day, will put the cornea under greater stress than a patient with a weekly wear schedule of 6 days and only 12 hours per day.

Chronic hypoxic stresses are even greater when SCLs are worn on a schedule that includes overnight wear (EW). This is because of the corneal oedema that results from a two-thirds reduction in oxygen availability in the closed eye. In EW, the cornea is exposed to a reduced oxygen level during the day (open eye) and a much reduced level during sleep (closed eye), i.e. the cornea is always either hypoxic (open eye) or more hypoxic (closed eye) whenever a lens is worn.

Key factors involved in SCL-induced corneal oedema are the:

- Water content of the material.
- Lens thickness.

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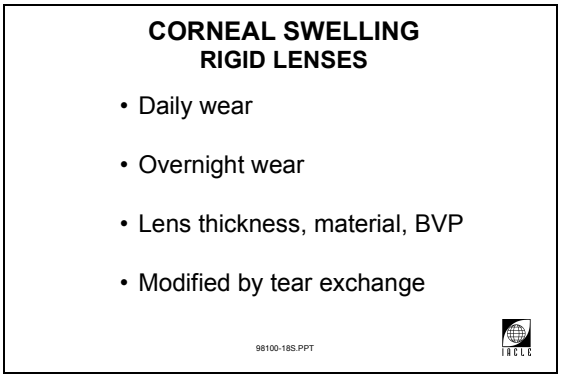

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Corneal Swelling with RGP lenses

RGP lenses do not impede the supply of oxygen to the cornea as significantly as do conventional SCLs. This is due to:

- Higher oxygen permeability of RGP materials.
- Lens design features, such as a smaller total diameter.
- Fitting characteristics, such as greater movement over the eye.

For a given lens material, it is important to consider lens design features. Thicker RGP lenses will supply less oxygen to the cornea than thinner lenses. Therefore, lens design features, as well as material oxygen permeability, must be carefully considered before a patient is fitted with RGP lenses for extended wear.

A major advantage of RGP lenses over SCLs is the significant exchange of tears that takes place with each blink. The supply of oxygenated tears in this manner supplements the transmission of oxygen through the lens. Tear exchange also has a role in the removal of sloughed-off cells from the cornea and some of the by-products of corneal metabolism.

The benefits of higher transmissibility, greater tear exchange and less than total corneal coverage, mean that corneal oxygenation is usually significantly higher with RGP lenses than other lens types. This results in less corneal oedema and more successful EW.

IV Effects of Hypoxia

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EFFECTS OF CORNEAL HYPOXIA

- Reduced aerobic glycolysis
- Lactate accumulation (stroma)
- Stromal acidosis
- Osmotic imbalance
- Oedema (swelling)
- Structural changes

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Effects of Corneal Hypoxia

Any reduction in the supply of oxygen to the cornea can have significant effects on normal metabolic activity. The effects are numerous and they range from mild to severe in their impact on the cornea.

Assessment of the effects of hypoxia on the eye provides a better understanding of corneal oxygen requirements. A number of *in vivo* studies have attempted to define the level of oxygen required by the cornea to prevent the occurrence of changes in structure or function.

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OXYGEN TENSION AND CORNEAL CHANGES

What level of pre-corneal oxygen tension is required to prevent changes to the normal human cornea ?

98100-9S.PPT

6L198100-9

Oxygen Tension and Corneal Changes

A large number of studies have examined the effect of changes to the pre-corneal oxygen tension and any resultant oedema of the corneal tissue.

Other effects from reduced oxygen supply to the cornea include changes to the:

- Epithelial mitotic rate.
- Density of nerve fibre endings.
- Sensitivity of the cornea.
- Corneal pH.

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EPITHELIAL MITOSIS

- 9% oxygen required to prevent:
  - suppression of mitosis
  - accumulation of lactate in the anterior chamber

98100-19S.PPT

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Oxygen Supply and Epithelial Mitosis

Hamano (1985) showed that when the oxygen level falls below 9%, a significant reduction in rabbit epithelial mitotic configurations occurs compared with that in the control eyes.

Glucose metabolism within the cornea is related to the amount of oxygen available to the tissue. It can be quantified in terms of the accumulation of lactate in the anterior chamber, which increases as the oxygen supply is reduced. To prevent the build-up of lactate, Hamano (1985) suggested that the level of oxygen must be 9% or higher.

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CORNEAL NERVE FIBRE ENDINGS

- Animal studies
- Hypoxia reduces the density of nerve endings
- 9-10% O_2 required

98100-20S.PPT

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Corneal Nerve Fibre Endings

Hamano (1985) also examined the effects of contact lens extended wear on the nerve fibre terminal endings in the rabbit.

Hypoxia caused the density of the terminal endings to decrease. His study determined that the oxygen level required to maintain the nerve fibres in their normal state is 9–10%.

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CORNEAL SENSITIVITY

- Increased touch threshold (reduced sensitivity) with:
  - prolonged eye closure
  - contact lens wear
  - hypoxia
- 8% O_2 required to maintain threshold

98100-21S.PPT

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Corneal Sensitivity and Contact Lens Wear

The cornea is the most densely innervated tissue in the body. This innervation protects the eye by making it highly sensitive and responsive to foreign bodies, abrasion, etc.

Millodot (e.g. 1984) has demonstrated that the cornea can be affected by a number of conditions to a point where the ability of the cornea to perceive a stimulus is severely reduced. He examined the touch threshold of the cornea using a Cochet-Bonnet aesthesiometer and found that hypoxia of the cornea resulted in an increase in the touch threshold value. Such an effect could make the cornea more susceptible to serious damage as the normal defensive mechanisms and reactions are absent or reduced.

A minimum of 8% oxygen is required to maintain corneal sensitivity at or near a normal level for the average person (Millodot and O'Leary, 1980).

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CORNEAL pH

- Fluorometric measurements
- Decreased pH with
  - closed eye
  - hypoxia
  - lens wear
- Bleb response
- To maintain normal pH:
  - epithelium: 21% O_2 required
  - endothelial/aqueous: 8% O_2

98100-42S.PPT

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Corneal pH and Contact Lens Wear

Bonanno and Polse (1987b) demonstrated that the corneal stromal environment becomes more acidic (lowered pH) in a range of circumstances including contact lens wear.

It is postulated that a reduction (Holden *et al*., 1985) or changes (Williams, 1986) in corneal pH cause the endothelial bleb response.

It has been estimated that in the rabbit, a Dk/*t* of greater than 300 ($x10^{-9}$) is required to prevent epithelial intracellular pH changes in the open eye (Giasson and Bonanno, 1994). This suggests an EOP of about 21% (calculation using the equation from Holden and Mertz, 1984, shows 25.5%, an impossibility under normal atmospheric circumstances). This implies unhindered access to atmospheric oxygen (see graph in slide 51). By way of contrast, a Dk/*t* of only 18 ($x10^{-9}$) is required to prevent aqueous pH changes (Giasson and Bonanno, 1994). This suggests an EOP of only about 8% is required.

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CLOSED EYE

- Reduced oxygen supply (palpebral conjunctiva)
- Partial lid closure effects?
- Decreased tear osmolality
- Increased CO_2 tension
- Decreased tear and stromal pH
- Increased corneal temperature (37°C)

98100-22S.PPT

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Effects of Eye Closure

When the eyelids are open, the majority of oxygen supplied to the cornea comes from the atmosphere. During sleep the lids are closed and significant changes take place in the ocular environment.

The main change is a reduction in the oxygen supply to the cornea. When the lids are closed the major source of supply becomes the palpebral conjunctival vessels. Some atmospheric oxygen may reach the cornea if the lids are only partially closed (lagophthalmos) and some may be supplied by the bulbar conjunctival vessels.

For the latter to be effective, some tear movement would be required. Rapid eye movement (REM) sleep could provide such movement but it is likely to result in only limited tear exchange.

Lid closure also causes a build-up in the levels of CO_2 in the cornea and results in an acidic pH shift in the tear film and stroma. The tear osmolality also decreases during sleep.

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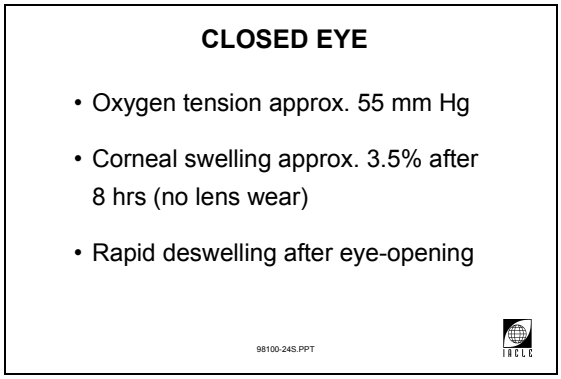

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Corneal Swelling with Eye Closure

Following eye closure, the anterior corneal oxygen tension falls rapidly to a steady state level of about 55mm Hg (for further details see slide 14 of this lecture). This hypoxic state causes the cornea to swell.

The literature suggest that the average amount of overnight swelling, i.e. swelling that occurs following 8 hours of eye closure when no lens is worn, is between 3 an 5.5% (e.g. 3%: Sweeney, 1991, 5.5%: Harper *et al.*, 1996, most other studies fall between these limits).

When the eyelids are opened following sleep, the corneal oedema resolves quickly and the corneal thickness returns to normal.

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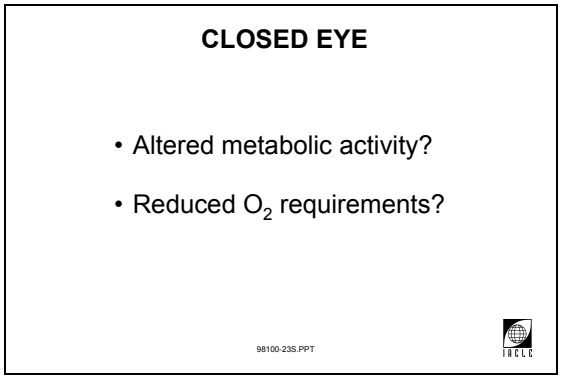

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Closed Eye

It is possible that, during periods of closed-eye sleep, there is a physiological reduction in metabolic activity that offsets the decreased availability of oxygen. If this is the case, the cells would presumably have a lower requirement for oxygen and could function effectively with less O_2.

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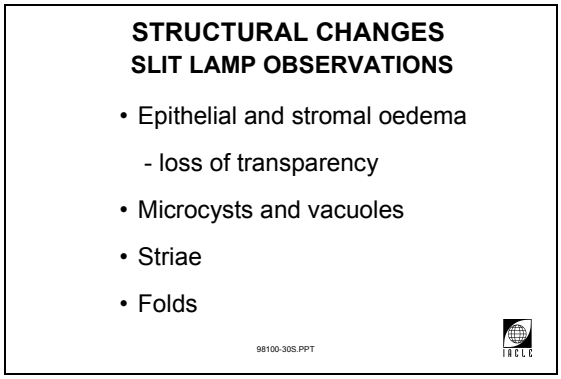

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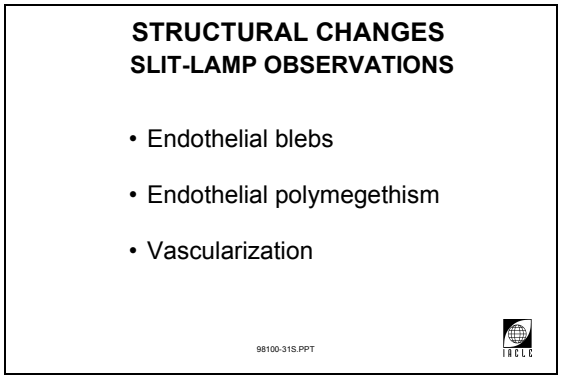

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Hypoxia and Structural Changes to the Cornea

Both short-term and chronic corneal hypoxia can result in significant changes in the structure and function of corneal tissue. In most cases, even subtle changes are visible with the slit-lamp biomicroscope.

All layers of the cornea can be affected by hypoxia. The contact lens practitioner must look for signs of hypoxia at each after-care examination (see Lectures 4.4 and 4.5 and Module 7).

65

MINIMUM OXYGEN REQUIREMENTS

CRITERION	MINIMUM O_2 (%)
• Corneal swelling	• DW: 9.9%, EW: 17.9%
• Epithelial mitosis	• 13.2%
• Epithelial healing	• 10.4%
• Corneal sensitivity	• 7.7%
• Glycogen depletion	• 5%
• Endothelial blebs	• 15-16.6%
• Nerve ending density	• 9-10%
• Nature's intention	• 20.946% ±0.002%

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Oxygen Requirements: Summary

The minimum oxygen level preferred by the eye is probably 20.95%, i.e. the level of oxygen generally available at sea level.

However, many people live the whole of their lives in environments in which less oxygen than this is available because of the altitude at which they live. Further, for about one-third of our lives, the anterior eye is exposed to only about one-third of this level of oxygen during eye closure and sleep.

Therefore, it may be more appropriate to think in terms of a desired minimum rather than an absolute minimum, despite the results of numerous studies offering minima based on study outcomes and the individual variation demonstrated. Such results are limited by study design, stimulus limitations, non-human subjects and, frequently, the lack of reality of the environment applied.

Rather than a 'minimum' level perhaps we should be using the term 'optimum', 'ideal', 'recommended' or as some authors use, 'critical' minimum level. Fatt used the phrase 'critical oxygen requirement' or COR.

Recommendations from some studies cannot be applied too rigorously since they exceed the levels available in many high-altitude regions of the world. The inhabitants of such regions thrive despite their 'oxygen deprivation'.

🕮 References

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Unit 6.2

(1 Hour)

Lecture 6.2: Corneal Oxygenation with Contact Lenses

Course Overview

Lecture 6.2: Corneal Oxygenation with Contact Lenses

I. Oxygen Permeability and Transmissibility

II. Measuring the Transmissibility of Contact Lenses

III. Classification of Contact Lenses

Lecture 6.2

(1 Hour)

Corneal Oxygenation with Contact Lenses

Table of Contents

I Oxygen Permeability and Transmissibility

1

CORNEAL OXYGENATION WITH CONTACT LENSES

98200-1S.PPT

6L298200-1

2

TEMPERATURE EFFECTS OF CONTACT LENSES

Open eye:

- With SCLs:
  - anterior surface 0.5°C cooler
- With RGP lenses (lower conductivity):
  - anterior surface >0.5°C cooler

Closed eye (cornea warms ≈3°C):

- No effect (RGPs and SCLs)
- No differences between surfaces

98200-36S.PPT

6L298200-36

3

TEAR FILM EFFECTS OF CONTACT LENSES

- Evaporation rates: SCLs ≈ RGPs
- CLs reduce BUT
- BUT:
  - RGPs - 4 to 6 s
  - SCLs - 4 to 10 s (H_2O content ↓, ∴ Dk/t↓)

98200-37S.PPT

6L298200-37

Oxygen Supply to the Cornea with Contact Lenses

As oxygen is required to maintain the cornea's normal metabolic activity and structural integrity, a key factor in the success of contact lens wear is the provision of an adequate supply of oxygen to the cornea.

All contact lenses reduce the amount of oxygen available to the cornea. In most cases, this reduction has only a minimal impact. However severe problems may occur in both the short and long-term should the oxygen availability fall well below the critical level required for on-going corneal health.

Contact lens wear exacerbates the phenomenon of lowered oxygen availability that occurs during eye closure. Further, corneal temperature has been shown to rise in the closed eye by about 3°C. Elevated corneal temperatures have been associated with increases in the anterior cornea's rate of metabolic activity (Freeman and Fatt, 1973). Therefore, the use of contact lenses in the closed eye presents a physiological challenge to the cornea because of the elevated temperature (largely not lens related) and reduced oxygen availability (a routine factor in eye closure exacerbated by the presence of contact lenses).

Temperature and Tear Film Effects of Contact Lenses

In the open eye, the presence of contact lenses has only a minimal effect. With SCLs, the lens' anterior surface is about 0.5°C cooler than the cornea underneath. With an RGP lens, the anterior surface is slightly cooler still as a result of the lens' lower thermal conductivity (Fatt and Chaston, 1980).

The tear film evaporation rate is believed to be about the same with both rigid and soft lens types (Hamano and Mitsunaga, 1982) with little difference attributable to their different surface areas. It should be noted however that evaporation rate and tear break-up time (BUT) are not synonymous, although the former is involved in the latter. Because of the instability of the tear film over contact lenses (Guillon *et al.*, 1989) the BUT of the pre-lens tear film is lower than the pre-ocular tear film. The pre-ocular BUT has been given as approximately 26 seconds while the RGP pre-lens BUT has been put at 4 to 6 seconds (Guillon *et al.*, 1989). The soft lens figures range between 4 and 10 seconds (see Figure 8 of Guillon *et al.*, 1990). The shorter BUTs for rigid lenses have been attributed largely to their smaller diameters and the significant 'draining' meniscus at their edges (Morris *et al.*, 1998).

In the closed eye it is probable that there are almost no temperatures changes induced by the presence of contact lenses and little difference in their front and back surface temperatures because tear evaporation is no longer a factor.

4

OXYGEN PERMEABILITY

- Intrinsic material property (resistance to gas flow)
- Permeability **P** = **Dk**

where:

D is the diffusion coefficient

k is the solubility coefficient of oxygen in a given material

98200-2S.PPT

6L298200-2

5

OXYGEN PERMEABILITY

- Independent of material thickness
- Dependent on temperature
- Calculated value

98200-3S.PPT

6L298200-3

Oxygen Permeability of Contact Lens Materials

Oxygen permeability (Dk) is an intrinsic physical property of the material from which a contact lens is fabricated. The diffusion coefficient (D) defines the speed of movement of the gas molecules within the material. The solubility coefficient (k) defines the number of oxygen molecules dissolved in the material (Fatt, 1992).

The permeability (Dk) varies directly with temperature, i.e. the higher the temperature, the greater the Dk. For most calculations a temperature of 34°C is used, as this approximates the corneal temperature in the open eye.

A contact lens material's oxygen permeability is a calculated value that provides a useful guide in clinical applications. In order to determine the Dk it is necessary to first do an *in vitro* test to measure the oxygen transmissibility (Dk/*t*) of a contact lens fabricated from it. This value is then multiplied by the thickness (*t*) to obtain the Dk.

A knowledge of a material's Dk allows the practitioner to design, or select a stock design of, a contact lens which will provide an adequate supply of oxygen to the cornea. This is especially the case when deciding on lens centre thickness because of its effect on lens transmissibility.

Where the oxygen permeability of a contact lens is to be measured, the lens must have parallel front and back surfaces, i.e. in the interests of having the influential central zone thickness uniform, the FOZR and BOZR should differ by the lens thickness only (FOZR–BOZR = t_c).

Powered lenses should be avoided because of the complexity introduced by the topographical variation in their thickness. However, it is noteworthy that a lens with *parallel* surfaces has a small amount of negative power.

Examples:

SOFT:	**RGP:**
FOZR : 8.9 mm	FOZR:7.9
BOZR : 8.8 mm	BOZR: 7.8
t_c : 0.1 mm	t_c: 0.1 mm
n : 1.399	*n* : 1.43
BVP : –0.37D	BVP: –0.49
(FOZR $_{Plano}$: 8.83	FOZR $_{Plano}$: 7.83)

I.A Units of Measurement

6

PERMEABILITY (Dk)
UNIT DERIVATION

$$\mathbf{D} = \frac{cm^2}{s} \;\&\; \mathbf{k} = \frac{mL_{O2}}{mL_{Lens} \times mmHg}$$

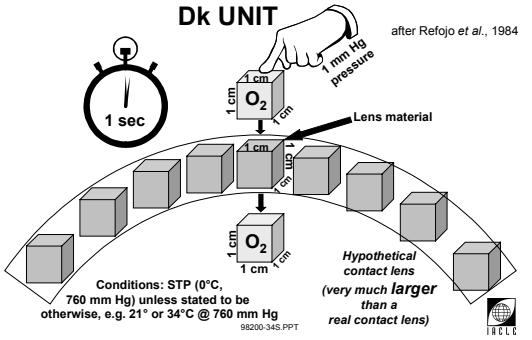
$$\therefore \mathbf{Dk} = \frac{cm^2}{s} X \frac{mL_{O2}}{mL_{Lens} \times mmHg}$$

$$\mathbf{Dk} = \frac{cm^2 \times mL_{O2}}{s \times mL_{Lens} \times mmHg}$$

98200-32S.PPT

6L298200-32

7



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8

OXYGEN PERMEABILITY

- Contact lens range: 0 - 300 x 10^{-11}
- Units:
  - (cm^2 x mL_{O2}) / (s x mL_{Lens} x mm Hg)

  or
  - (cm^2/s) x (mL_{O2} / [mL_{Lens} x mm Hg])
  - commonly, the $_{Lens}$ subscript is omitted

98200-4S.PPT

6L298200-4

Oxygen Permeability: Units

The unit of oxygen permeability (Dk) is derived from the diffusion coefficient D and the solubility coefficient k (slide 4).

- D, the diffusion coefficient, defines the velocity of oxygen gas molecules moving in the lens material. The 'velocity' of randomly moving gas molecules diffusing through a material is expressed in terms of an area per second, i.e. cm^2/s rather than the more conventional cm/s that applies to non-gaseous entities (Fatt, 1995).
- k, the solubility coefficient, defines the volume of oxygen that can be dissolved in the contact lens material (after Fatt, 1995). k is expressed in terms of the volume of oxygen, the volume of lens material and the pressure driving the oxygen into, and through, the lens material, i.e. $mL_{O2}/(mL_{Lens} \times mm\ Hg)$.
  (*Note: millilitres (mL) and cubic centimetres (cm^3) are used interchangeably here. However, they are not exactly the same due to a minor error in the early definition of the litre. Strictly, 1 litre (L) is the volume occupied by 1 kilogram (kg) of pure water at the temperature of its maximum density (approximately 3.96°C). More accurate measurements made subsequently, showed that this volume was actually 1.000028 cubic decimetres ([10 cm]^3). Therefore, one mL is fractionally smaller than 1 cm^3 but the inaccuracy is only of the order of 1 in 36,000 (Jerrard and McNeill, 1992).*

A graphical concept of the Dk unit is presented in slide 7. In this slide 1 cm^3 of oxygen is being driven through 1 cm^3 of polymeric contact lens material (any type) by a positive partial pressure of oxygen (pO_2) difference across the lens of 1 mm of mercury pressure in 1 second under a stated set of environmental conditions.

While the atmospheric pressure is usually assumed to be 760 mm Hg, the temperature must be stated because the Dk is temperature sensitive. This is largely because the level of gas molecule activity (agitation) increases with temperature. Increased activity increases the success rate of gas molecules entering, and eventually passing through, a lens polymer. Slide 7 is a purely theoretical representation for illustration purposes only. Real contact lenses contain nothing like the volume of polymer implied in slide 7, nor are driving pressures as low as 1 mm Hg; oxygen volumes as high as 1 cm^3; and the time involved as short as 1 second.

Estimates of human corneal oxygen consumption vary between 1.6 and 10.9µL per cm^2 of cornea per hour (Efron and Brennan, 1992). Realistic measures result in an exponent of 10^{-11} being

appended to the Dk unit (slide 10). Furthermore, in many instances in which Dk units are presented, the lack of brackets or statements of mathematical processing precedence lead to potential confusion as to which components of the unit form the numerator (top) and which form the denominator (bottom).

Recently, Alvord *et el*. (1998) have suggested that an historic unit of oxygen permeability, the barrer, be used as an alternative to the widely used, but somewhat cumbersome 10^{-11} (cm^2 x mL_{O2})/(s x mL_{Lens} x mm Hg). They argue that by using the barrer for permeability and the more traditional unit for transmissibility, i.e. 10^{-9} (cm x mL_{O2})/(s x mL_{Lens} x mm Hg), confusion between the two can be reduced. To date, the barrer has not gained wide acceptance.

9

OXYGEN TRANSMISSIBILITY

- Based on material *permeability* (Dk)
- Related to material *thickness* (*t*)
  - transmissibility = Dk/*t*
- Relevant clinically

98200-5S.PPT

6L298200-5

10

TRANSMISSIBILITY (Dk/*t*)
UNIT DERIVATION

$$\mathbf{Dk} = \frac{cm^2 \times mL_{O2}}{s \times mL_{Lens} \times mmHg}$$

$$\therefore \mathbf{Dk/t} = \frac{cm^2 \times mL_{O2}}{s \times mL_{Lens} \times mmHg \times cm}$$

$$\mathbf{Dk/t} = \frac{cm \times mL_{O2}}{s \times mL_{Lens} \times mmHg}$$

98200-33S.PPT

6L298200-33

11

OXYGEN TRANSMISSIBILITY

- Contact lens range: 0 - 200 x 10^{-9}
- Units:
  - (cm x mL_{O2}) / (s x mL_{Lens} x mm Hg)
  - or
  - (cm/s) x (mL_{O2} / [mL_{Lens} x mm Hg])
  - commonly, the $_{Lens}$ subscript is omitted

98200-6S.PPT

6L298200-6

Oxygen Transmissibility of Contact Lenses

The oxygen transmissibility of a contact lens is a physical property of the actual lens, i.e. a property of both its material and its thickness. It is equal to the material's oxygen permeability divided by the lens thickness, i.e. Dk/*t*.

The exponent 10^{-9} is appended to the unit of transmissibility because lens thickness is expressed in centimetres (cm), e.g. a thickness of 0.1 mm is 0.01cm. It is normally specified at a specific temperature, usually 34°C, sometimes 35°C.

The permeability, and therefore the transmissibility, are temperature dependent and are related directly, i.e. the higher the temperature, the higher the Dk and therefore the higher the Dk/*t*. Because of the greater relevance of an on-eye temperature, such figures are probably more useful clinically. Alternatively, an *in vitro* laboratory temperature may be quoted. This is usually 20° or 21°C.

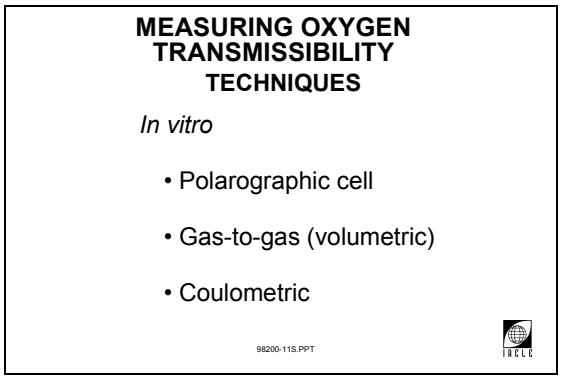
Clinically, oxygen transmissibility is more useful than oxygen permeability. It relates directly to on-eye issues as it includes lens thickness.

Commonly, the transmissibility of a contact lens series is given for a lens BVP of –3.00 D, a BVP that has become something of an industry standard.

However, it is important to realize that the value stated varies according to lens BVP because of the latter's effect on lens thickness. A lens of plus power has significantly *less* transmissibility at its centre than does a minus powered lens. The Dk/*t* of a minus lens has little relevance to a plus lens, especially one of significant plus power.

II Measuring The Transmissibility of Contact Lenses

12



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Techniques for Measuring Oxygen Transmissibility

The measurement of a contact lens' oxygen transmissibility is usually an *in vitro* laboratory process.

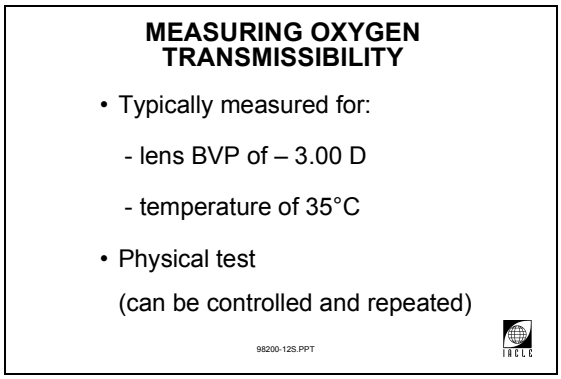
Many of the methods are well established with the exception of the coulometric technique, a two-chamber technique, developed more recently by Winterton *et al.* (1987).

The volumetric (gas-to-gas) method, while probably the oldest method in general science, was not applied to contact lenses until after the use of polarographic sensors had become common in eye and contact lens measurements (Haberich, 1966 in Fatt, 1978, Fatt, 1991).

Possibly because of simplicity and economy, polarographic methods are still the most common although there seems to be general agreement on the merits of the coulometric system. This is partially due to its negligible boundary layer resistance, a correction for which is required with other methods.

Despite the methods and apparatus available, and the refinements made to the techniques over time, none can account for batch-to-batch differences between materials (Winterton *et al.*, 1987). Most of the 'differences' in the Dks reported for similar materials can be attributed to the use of different techniques, apparatus and procedures (Holden *et al.*, 1990).

13



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Measurement of Oxygen Transmissibility

The use of rigorous scientific methods for the determination of lens Dk/*t* means that measurements can be performed by other workers at other locations using a variety of equipment in the expectation that very similar results will be achieved. A review of the issues has been published (Holden *et al.*, 1990).

Commonly, the oxygen transmissibility is measured for lenses of −3.00D BVP at a temperature of 35°C, an approximation to on-eye temperature.

II.A The Polarographic Technique

14

MEASURING OXYGEN TRANSMISSIBILITY
POLAROGRAPHIC CELL TECHNIQUE

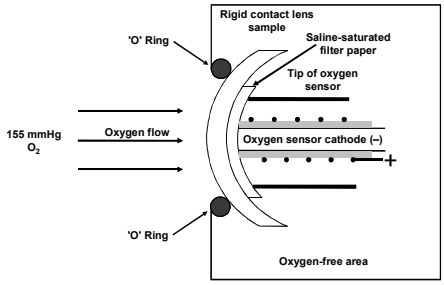
- Sensor in measuring cell contains:
  - anode (+)
  - cathode (–)
  - electrolyte
- Contact lens becomes the 'membrane'
- Controlled humidity and temperature

98200-13S.PPT

6L298200-13

15

POLAROGRAPHIC CELL



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Polarographic Cell Technique

Fatt and St Helen (1971) applied the polarographic technique to the determination of the transmissibility of contact lenses.

The basics of the polarographic oxygen sensor have been dealt with in detail in Lecture 6.1. When used for transmissibility measurements, the membrane of the polarographic oxygen sensor is replaced by the contact lens to be tested. Essentially, the other aspects of the sensor are as described previously.

A contact lens is mounted in the apparatus so that its front surface is exposed to the atmosphere. The oxygen sensor is separated from the atmosphere by the lens covered by an aqueous film. The combination of lens and barrier film acts as a limitation to the flow of oxygen *from* the pre-lens environment *to* the platinum cathode of the oxygen sensor (slide 15).

16

POLAROGRAPHIC CELL TECHNIQUE

- Oxygen passes through the lens into the sensor's electrolyte
- Sensor current is proportional to the amount of oxygen available at the cathode
- Oxygen flux j from Fick's & Henry's laws:

$$j = Dk/t \times \Delta(pO_2)$$

98200-14S.PPT

6L298200-14

17

POLAROGRAPHIC CELL TECHNIQUE

- Permeability calculated from:
  - thickness of the lens (*t*)
  - current required to reduce O_2 (i)
  - partial pressure of O_2 (pO_2)
  - cell constant (C)

$$Dk = \frac{C \times t \times i}{pO_2}$$

98200-15S.PPT

6L298200-15

Chemical Reaction in the Polarographic Cell

Measurement of the oxygen transmissibility and material permeability of contact lenses is based on both Fick's (diffusion) and Henry's (solubility) laws. Fick's law relates the steady-state flux (passage) of a gas across a plane to the concentration gradient across that plane.

Henry's law relates the level of dissolved oxygen in a substance to the solubility of oxygen in that substance and the partial pressure of oxygen (pO_2) within the substance.

$$j = Dk/t \times \Delta(pO_2)$$

The oxygen flux (j) refers to the amount of oxygen that flows through a unit area (e.g. cm^2) of material in a unit time (e.g. second). $\Delta(pO_2)$ is the difference between the partial pressures of oxygen on either side of the lens. Dk/*t* is the lens transmissibility. The Dk, i.e. the material permeability, is usually considered as a whole rather than the product of the diffusion and solubility coefficients. The lens sample thickness is *t*.

The electrochemistry of the oxygen sensor has been described previously (Lecture 6.2, Section II.A). Importantly, any oxygen at the cathode is converted into hydroxyl ions (OH^-) thereby reducing the oxygen level to zero at the cathode's surface. If the oxygen flux is great, it is conceivable that a cathode of small surface area can not convert

oxygen to hydroxyl ions fast enough. In this case a larger cathode is required. It is possible that different sensor designs may be required for different ranges of lens transmissibility to overcome such difficulties.

A further complication of polarographic sensors is that their behaviour is never ideal, e.g. theoretically when the pO_2 is zero, the sensor current should also be zero. The actual current measurable at zero pO_2 is termed the 'dark current'. A partial answer to this difficulty is to analyze data over a range of pO_2s significantly above zero.

Using an appropriate design, an O_2 concentration gradient from 155mm Hg at the anterior surface to zero at the cathode can be established. Oxygen molecules that diffuse down this gradient are eliminated by reduction at the cathode and the current formed at the point of equilibrium is proportional to the rate of oxygen flow (the flux) across the contact lens.

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POLAROGRAPHIC CELL TECHNIQUE

- Utilizes finished contact lens
- Potential errors include:
  - boundary layers
  - edge effects
  - lens thickness
  - environment
  - cell integrity
  - calibration

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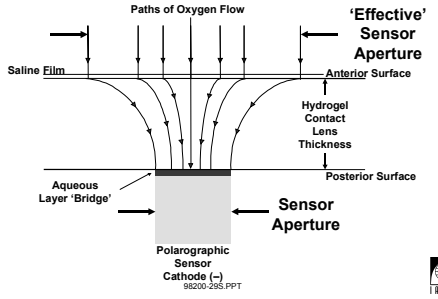
19

EDGE EFFECT



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Polarographic Cell Technique Considerations

The polarographic cell technique was the first method used to calculate the oxygen permeability and transmissibility of contact lenses (Fatt and Chaston, 1982). As the method involves a number of potential errors, steps are required to minimize their magnitude if reliable results are to be produced. The discrepancies in the results published in the past can be attributed largely to a failure to account for these potential errors, or simply ignorance of them.

If a contact lens has a residual layer of saline or water on the surface, the resistance to the diffusion of oxygen through the lens 'system' is increased, as the surface water layer acts like another lens in series with the lens being measured. The polarographic cell is then evaluating the diffusion of oxygen through both the water layer and the contact lens.

Another significant source of error is the so-called 'edge effect' (see slide 19). The edge effect is due to the difference in the surface area of the lens through which oxygen can diffuse to the sensor (upper lens surface), and the actual aperture of the sensor itself (in contact with the lower lens surface). This effect is greater in thicker samples and materials of higher permeability. Effectively, the sensor accepts oxygen from a larger area of the lens than the physical diameter of the sensor would suggest.

The diffusion pathway is believed to be funnel shaped (slide 19). The magnitude of the error attributable to the edge effect may be as large as a 25% overestimate of the oxygen permeability.

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POLAROGRAPHIC CELL TECHNIQUE
DISADVANTAGES

- Not suited to highly permeable, non hydrogel materials
- Over-estimates values for RGP lenses
- Variability among investigators

98200-17S.PPT

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Disadvantages of the Polarographic Cell Technique

Due to the static nature of the test, this technique does not work well with highly permeable, non-hydrogel materials like siloxane elastomers. Neither the electrolyte in the cell nor the saline (or water) film on the front of the lens is stirred. Therefore, a stagnant boundary layer develops at the liquid-material interface within the sensor and on the lens. Due to the inherent potential errors with the polarographic cell, the technique is not optimal for measuring the oxygen transmissibility of RGP lenses.

Their rigidity and steeper curvature are two potential difficulties. Further, a highly porous electrolyte 'bridge' between the lens and the cathode needs to be created with cigarette paper, filter paper or lens (optical instruments) cleaning tissue. Generally, the results over-estimate the Dk/*t* unless all errors in the method are controlled (Brennan *et al*. 1986). Variability in the technique has produced significant differences in the results published by various investigators. It is important to know whether the quoted lens Dk/*t* was measured using methodology that corrects for potential errors such as edge effects. Not surprisingly, marketing efforts are more likely to focus on the higher results regardless of the method of their derivation.

II.B The Gas-to-Gas Technique

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MEASURING OXGEN TRANSMISSIBILITY
GAS-TO-GAS

- Two environmental chambers
  - pure oxygen
  - differential pressures
- Constant temperature (35°C)
- Pressure sensor in each chamber

98200-18S.PPT

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Measuring Oxygen Transmissibility: Gas-to-Gas

In this method of measuring oxygen transmissibility, the contact lens under test separates two self-contained chambers (slide 22) (Benjamin, 1994). The environment within each chamber is controlled.

Typically, the anterior chamber of the apparatus contains pure pressurized oxygen maintained at three atmospheres (2280 mm Hg), while the posterior chamber contains pure oxygen at one atmosphere (760mm Hg). The temperature is usually set to 34° or 35°C with controls to prevent significant deviations from this figure.

A transducer in each chamber permits accurate measurement and control of the chamber pressures. The pressure in the closed posterior chamber increases as the oxygen flows *from* the higher pressured anterior chamber through the lens.

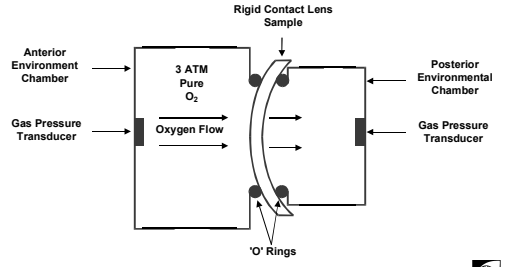
Because the volume in the posterior chamber is constant, the rate of oxygen flow through the contact lens can be calculated from the recorded rate of pressure increase using the standard 'ideal gas equation':

$$PV = nRT$$

P = pressure n = number of moles of gas
R = the gas constant T = temperature in °K
V = volume

22

GAS-TO-GAS TECHNIQUE



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23

MEASURING OXYGEN TRANSMISSIBILITY
GAS-TO-GAS TECHNIQUE

- Constant pressure in anterior chamber
- Gas flow through the lens alters the pressure in the posterior chamber
- No boundary layer or edge effects
- Can be used for any gas

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This technique has an advantage over others in that it produces no boundary layer or edge effects. It can also be easily applied to gases other than oxygen, e.g. carbon dioxide.

More recently Fatt (1991) described a gas-to-gas method of measuring the oxygen transmissibility of RGP and siloxane contact lens materials. His method avoided any boundary layer effects by utilizing a gaseous phase on either side of the lens under test. A small edge effect was still present. Fatt recommends this method for RGP and siloxane materials transmissibility measurements.

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GAS-TO-GAS TECHNIQUE
DISADVANTAGES

- Not suitable for hydrogels
  - pressure differential too great
  - hydrogels too elastic
  - hydrogels have low burst strength

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Gas-to-Gas Technique: Disadvantages

Due to the large pressure difference between the two chambers, and the elastic nature and lower burst strength of hydrogel materials, it is not possible to adapt this technique to the measurement of the gas transmissibility of hydrogels.

II.C The Coulometric Technique

25

MEASURING OXYGEN TRANSMISSIBILITY
COULOMETRIC TECHNIQUE

- Two 'environmental chambers'
  - oxygen
  - inert gas
- Coulometric sensor
- Oxygen flow through lens is measured
- Water saturated oxygen or liquid reservoir required for hydrogels

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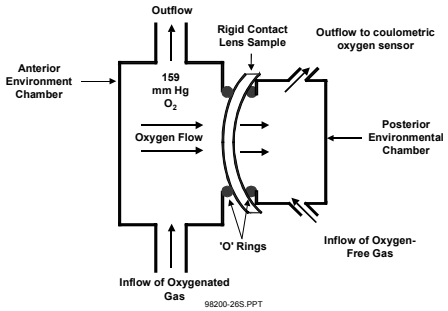
COULOMETRIC TECHNIQUE



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Coulometric Technique

In the coulometric technique, a contact lens separates two open chambers (slide 26). The anterior chamber is filled with a humidified oxygen-containing gas. In the posterior chamber, an oxygen-free gas flows across the back surface of the contact lens and is directed toward a coulometric sensor (Benjamin, 1994). The coulometric sensor is basically a polarographic sensor. However in this scheme it is not in contact with the lens under test.

The flow of oxygen is *from* the anterior chamber to the posterior chamber. Oxygen reaching the posterior chamber after passing through the lens is swept along, and mixes, with the inert carrier gas towards the coulometric oxygen sensor. The sensor generates an electrical current that is proportional to the concentration of oxygen in the inert carrier gas.

In this way, the rate of oxygen flow is detected and recorded. From the results, the oxygen permeability and transmissibility can be determined (Winterton *et al.,* 1987, Winterton *et al.*, 1988).

Intra-lot standard deviations of <2% are claimed by the developers (Winterton *et al.*, 1987).

Importantly, there are only small stagnant film (boundary layer) effects, no significant edge effects and no need for electrolyte bridges in an oxygen sensor.

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COULOMETRIC OXYGEN SENSORS CHEMISTRY

Cathode (– ve, Carbon [graphite]):

$4\ e^- + O_2 + 2H_2O \rightarrow 4OH^-$

Anode (+ ve, Cadmium [nickel-cadmium]):

$2\ Cd + 4OH^- \rightarrow 2Cd(OH)_2 + 4e^-$

e = electron

Overview:

$\mathbf{2Cd + O_2 + 2H_2O + 4OH^- \rightarrow 2Cd(OH)_2 + 4OH^-}$

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The coulometric sensor is much larger (9-10X) than a polarographic sensor and, unlike the polarographic sensor, uses a graphite cathode and a sintered nickel-cadmium anode in a potassium hydroxide electrolyte. However, the electrochemistry (slide 27) is similar to that of the platinum-silver/silver chloride electrode pair described previously (Lecture 6.1, Section II.A).

The dry inert carrier gas used is 98% nitrogen and 2% hydrogen while the test gas is air or an oxygen/nitrogen mixture that approximates it. All gases are humidified to saturation before application. All details from Winterton *et al.*, 1987.

Winterton *et al.* (1988) have shown that surface wettability has an effect on the lens transmissibility. They postulated this was due to a boundary layer effect at the lens surface, and the better the wettability the less the effect, due to more intimate contact with the lens surface being established.

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COULOMETRIC TECHNIQUE ADVANTAGES

- Posterior lens surface is exposed
- Little or no boundary layer effects
- No edge effects
- More accurate than polarographic techniques with RGP contact lenses

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Coulometric Technique: Advantages and Disadvantages

The main advantage of the coulometric technique is its ability to measure the oxygen transmissibility of RGP lenses accurately. Minor (Winterton *et al.*, 1988) or no boundary or edge effects are created in this system (Benjamin, 1994).

For the measurement of hydrogel materials a reservoir of saline solution or a water-saturated gas must be in contact with the front surface of the lens. The use of a saline reservoir creates an anterior boundary layer, which must be factored into the calculation of any SCL oxygen transmissibility value.

The polarographic cell method is the favoured option for the measurement of the oxygen transmissibility of hydrogel lenses.

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COULOMETRIC TECHNIQUE DISADVANTAGES

Requires:

- Specific gas (e.g. oxygen) sensor
- Anterior aqueous reservoir for hydrogels

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III Classification of Contact Lenses

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TRANSMISSIBILITY CLASSIFICATION
SOFT LENSES

Low < 12

Mod 12 - 25

High > 25

98200-24S.PPT

6L298200-24

Classification of Oxygen Transmissibility of Hydrogel Lenses

Current classifications of hydrogel lenses do not take into account the new highly oxygen permeable polymers (siloxane and fluoro-siloxane hydrogels).

Development of new polymers for use as soft contact lenses will see a reclassification of the transmissibility index. Some of the new polymers provide oxygen transmissibilities in the range of 100 - 180 x 10^{-9} units.

Even the most highly oxygen transmissible hydrogel lenses available currently can only just meet the Holden and Mertz (1984) criteria for daily wear due to the limited ability of oxygen to pass through their significant water contents.

Current hydrogels range in water content from 37.5% to 79%. Their water content has become a barrier to further improvements in material permeability since even the impossible water content of 100% will not meet the Holden-Mertz (1984) criterion for extended wear ($Dk/t \geq 87 \times 10^{-9}$).

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TRANSMISSIBILITY CLASSIFICATION
RGP LENSES

Low < 25

Mod 25 - 50

High > 50

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6L298200-25

Classification of RGP Lens Oxygen Transmissibility

The vast majority of RGP lenses provide substantially higher levels of oxygen to the cornea than do conventional hydrogel lenses. Some of the more highly permeable materials are capable of meeting the Holden-Mertz extended wear criterion, which is itself a minimum recommendation.

🕮 References

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Unit 6.3

(1 Hour)

Lecture 6.3: Contact Lens Characteristics and Oxygen Transmission

Course Overview

Lecture 6.3: Contact Lens Characteristics and Oxygen Transmission

I. Corneal Oxygen Requirements

II. RGP Permeability and Transmissibility

III. SCL Permeability and Transmissibility

IV. Corneal Swelling with Contact Lenses

Lecture 6.3

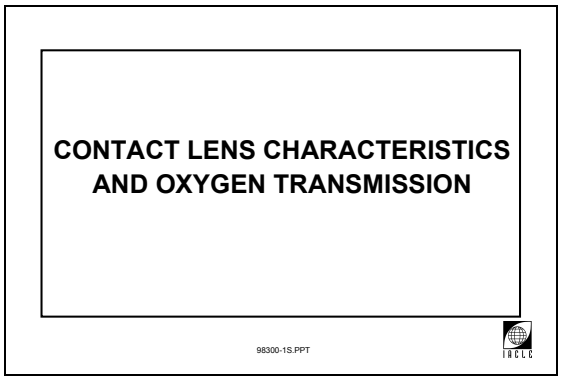
(1 Hour)

Contact Lens Characteristics and Oxygen Transmission

Table of Contents

I Corneal Oxygen Requirements

1



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Contact Lenses and Oxygen Transport to the Cornea

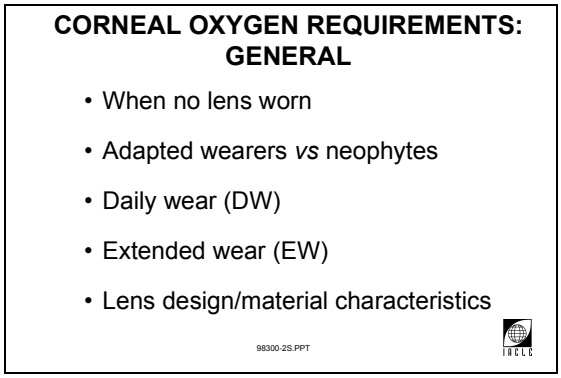
A major goal of contact lens material research and development is to increase material oxygen permeability to boost the levels of oxygen available to the cornea during lens wear.

Breakthroughs in the polymer chemistry of hydrogels, siloxane elastomers and rigid gas permeable lens materials have resulted in contact lenses that offer a wide range of oxygen transmissibilities.

As advances yield higher oxygen performances, older materials that offer no special advantages or characteristics are deleted from product lines. Particularly in the case of RGP lens materials, this means that current product lines range from average to high performance rather than low to average.

Issues not related to oxygen which may help a less permeable material remain attractive to users include economic pricing, ease of manufacture, stability of parameters when worn and surface wettability *in situ.*

2



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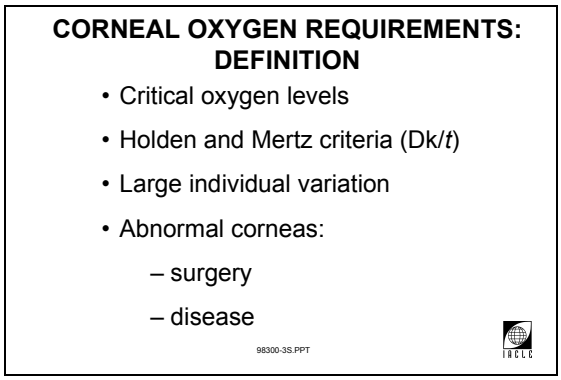
Corneal Oxygen Requirements: General

To understand the effects of contact lenses on the structure and function of the cornea, it is necessary to consider its normal oxygen requirements under a variety of conditions both with and without lenses.

The major questions are:

- What is the minimum level of oxygen required by the cornea to maintain normal metabolic activity?
- Does the corneal oxygen demand change with lens wear?
- What effects do the different wear modalities have on the cornea's need for oxygen?
- How do the variables of lens material and design affect corneal oxygen supply during lens wear?

3



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Corneal Oxygen Requirements: Definition

Many researchers have attempted to measure and define the minimum amount of oxygen the cornea needs to maintain successful contact lens wear.

These studies have investigated numerous phenomena associated with contact lens wear including:

- Corneal swelling.
- Structural changes.
- Biochemical changes.
- Alterations to cell reproduction.
- Changes in corneal sensory behaviour.

Polse and Mandell (1971) conducted one of the earliest studies that attempted to define the critical oxygen requirements of the cornea. The major study in this field is that of Holden and Mertz (1984). Holden and Mertz attempted to define the minimum contact lens oxygen transmissibility required to meet the needs of various modes of contact lens wear. Their assessment of need was based on the levels of corneal swelling (oedema) measured by optical pachometry.

Their study, which was derived from a series of large clinical studies, provided valuable information on the average human corneal oxygen requirement.

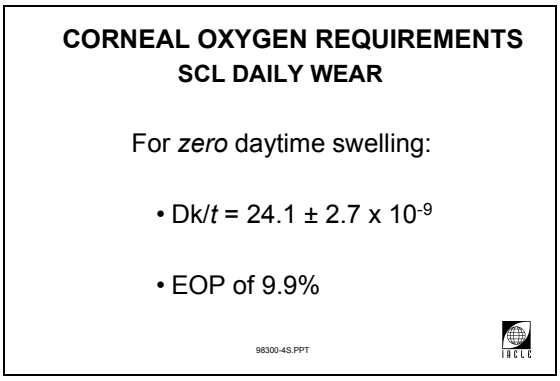
However, it is important to note that because of significant individual variation, many contact lens wearers require more corneal oxygen than the average to maintain normal corneal function, while others may need less. Only wearers in the former category are a concern clinically.

Consideration must also be given to the oxygen needs of the cornea that has undergone surgery or been affected by a disease process (Holden et al, 1980).

More details on the critical oxygen requirements according to various physiological criteria, and the effects of hypoxia on the cornea, are given in Lecture 6.1, Section IV.

I.A Oxygen Requirements to Prevent Corneal Swelling

4



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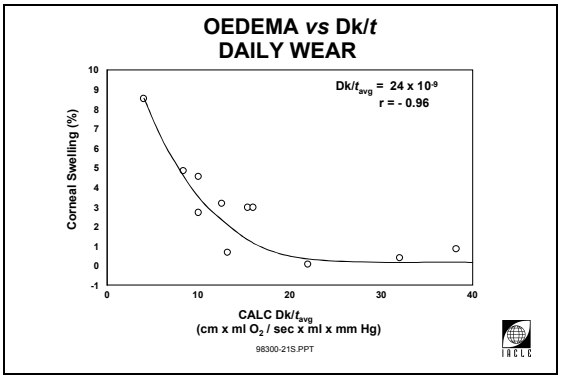
Oxygen Requirements During SCL Daily Wear

Physiologically, the ideal SCL, when worn on a daily wear (DW) basis, should cause zero corneal oedema (swelling). To minimize its impact on the average cornea, the oxygen transmissibility of a SCL should be 24.1 ± 2.7 x 10^{-9} units based on the study of Holden and Mertz (see slides 4 and 5).

Alternatively, this oxygen requirement can be expressed in terms of the equivalent oxygen percentage (EOP) required at the corneal surface. For a SCL to induce zero swelling during DW, the EOP value should be 9.9% for the 'average' cornea.

5



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6

CORNEAL OXYGEN REQUIREMENTS
SCL EXTENDED WEAR

For *zero* residual swelling at eye closure:

- Dk/*t* = 34.3 ± 5.2 x 10^{-9}
- EOP of 12.1%

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Oxygen Requirements During SCL Extended Wear: Zero Residual Swelling

Following overnight wear of an SCL, the cornea swells by an amount which is related to the oxygen transmissibility of the material. After eye opening, the cornea begins to thin as its metabolic activity increases due to the higher availability of oxygen.

Holden and Mertz (1984) determined the SCL oxygen transmissibility required to permit the cornea to return to baseline corneal thickness (no residual daytime oedema) prior to the next period of eye closure, when lenses are worn on an extended wear basis.

According to their calculations, the oxygen transmissibility of an SCL must be 34.3 x 10^{-9} units or an EOP of 12.1%. This Dk/*t* value limits the permissible overnight corneal swelling to approximately 8.0%.

7

CORNEAL OXYGEN REQUIREMENTS
SCL EXTENDED WEAR

For *overnight* oedema = 4.0%

- Dk/*t* = 87.0 ± 3.3 x 10^{-9}
- EOP of 17.9%

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Oxygen Requirements During SCL Extended Wear: Maximum of 4% Overnight Swelling

Physiologically, the ideal SCL for extended wear is one that produces no more corneal swelling than a non-lens wearing eye following eight hours of sleep. The normal, no-lens overnight corneal swelling is of the order of 4.0% (the literature provides figures between 3% and 5.5%).

To achieve this, the oxygen transmissibility of an SCL must be 87.0 x 10^{-9} units (slides 7 and 8) or an EOP of 17.9% (Holden and Mertz, 1984).

More recently, this area has been re-examined by Harvitt and Bonanno (1999) using a model incorporating their finding that increased corneal oxygen consumption results from corneal acidification (Harvitt and Bonanno, 1998).

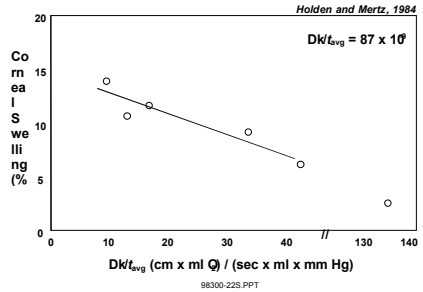
For humans they found the minimum lens transmissibility that would deliver oxygen to the basal epithelial cells was 23 (X10^{-9}) for the open eye and 89 (X10^{-9}) for the closed eye. These figures agree very closely with Holden and Mertz (1984) estimates (24 and 87 respectively).

To prevent anoxia across the entire corneal thickness, Harvitt and Bonanno (1999) suggested transmissibilities of 35 (X10^{-9}) for the open eye and 125 (X10^{-9}) for the closed eye are required.

These figures represent a new 'high-water mark' in the estimates of the oxygen minima required to prevent hypoxia-related adverse corneal responses. The latest generation of RGP and siloxane-containing hydrogel materials can deliver these levels, whereas previous generation materials could not.

8

OVERNIGHT OEDEMA *vs* Dk/*t*
EXTENDED WEAR



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II Oxygen Supply to The Cornea

9

DYNAMIC OXYGEN SUPPLY

- Post-lens tear film exchange rates:
  - SCL 1% per blink
  - RGP 15 - 20% per blink
- Blinking
  - rate
  - quality (completeness)
- RGP *vs* SCL fitting characteristics

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Lens Dynamics and Oxygen Supply

Measurement of oxygen transmissibility provides an indication of the amount of oxygen that will pass through the bulk of the lens to the cornea.

However, it is also important to assess the tear exchange behind a contact lens, as oxygen also reaches the cornea in the tear fluid. This issue is covered in Lecture 6.1, Section I.B.

Soft lenses have only minimal tear exchange with each blink due to their large total diameter and conformity with the corneal and conjunctival surface shapes (Polse 1979). Parrish and Larke (1981) also showed that not only was the SCL tear pump 'small' but that it also varied slightly with lens fit. Consequently, the oxygen transmissibility of the contact lens is the principal determinant of the supply of oxygen to the cornea for SCLs.

On the other hand, rigid gas permeable (RGP) lenses can supply significantly more oxygen to the eye than SCLs via the exchange of tears that occur with each blink. Their greater tear exchange is due to their smaller total diameter, and greater on-eye movement.

It is estimated that 10-20% of the tear film behind an RGP lens is exchanged for fresh, oxygenated tears following a blink.
Therefore, in RGP lens wearers the blink rate, the completeness of each blink and the lens transmissibility are all important determinants of corneal oxygenation.

10

GAS MOVEMENT THROUGH LENSES

- Composition of polymer
- Temperature effect
- Partial pressure of gas at lens surface
- Lens thickness
- Boundary layer effect

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Gas Movement Through Contact Lenses

Five major parameters dictate the oxygen permeability of a contact lens material.

The most important of these parameters is the composition of the polymer from which the lens is fabricated.

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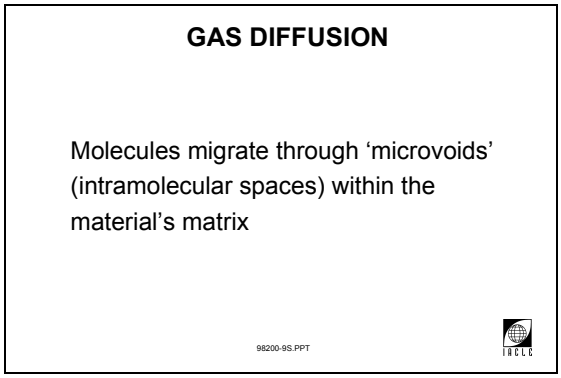

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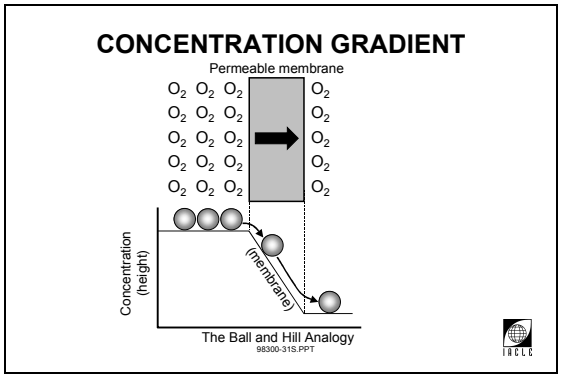

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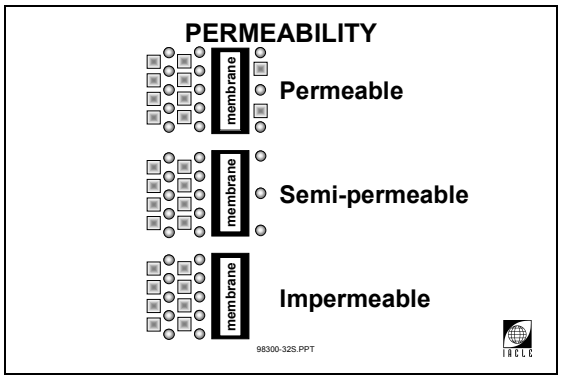

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Gas Diffusion

Diffusion is the process by which gas molecules (or molecules of other substances), move within another gas (or liquid or solid) to fill uniformly all of the available space. This is made possible by the continuous random movement exhibited by all molecules whose temperature is above absolute zero, i.e. 0°K or –273.15°C. At absolute zero (only approximated but not realized to date) all motion would cease and any diffusion would be suspended.

When the concentration of a substance, or substances, is higher in a particular region of an enclosed space, the number of molecule collisions in this region is greater. This leads to a tendency for the molecules to distance themselves uniformly within the space, over time. In this situation, the least number of collisions occur, i.e. a state is reached in which the least amount of energy is expended on collisions.

Similarly, when a concentration gradient exists across a membrane, there will be a tendency for the molecules to pass from the region of higher concentration towards that of lower concentration until such time as the concentrations are equalized (slide 12).

However, some membranes can resist (limited permeability) or even prevent (impermeable) the transfer of molecules. Some are somewhat selective in that they allow some molecular species to pass while denying passage to others, i.e. they are said to be semi-permeable (slide 13).

If the membrane is permeable to the substance in question, molecules do not only pass in one direction, rather the net effect is as if the flow is unidirectional. This is because the activity of molecules on the lower concentration side results in some of the molecules travelling against the concentration gradient.

However, for each molecule travelling against the gradient, many more travel with the gradient because of the greater collision frequency in the space of greater concentration. This results in a net flow from the space of higher concentration to that of the lower concentration.

Ultimately, an equal number of molecules are passing in either direction, i.e. a state of equilibrium is reached. This can only occur when the concentrations on either side of the membrane are equal.

Gas molecules colliding with the membrane molecules can result in a failure to penetrate the membrane at all, or a more circuitous route being taken because of deflection or reflection of the molecule from its original trajectory.

In summary: when a membrane separates a mixture of gases, the molecules in greater concentration are propelled by their kinetic energy (molecular movement), and the resulting collisions,

into and then across the membrane to the side with the lower concentration (after Refojo *et al.,* 1984).

The transfer rate depends largely on the concentration gradient (differences in partial pressures), temperature (affects level of molecular agitation) and the properties of the membrane itself.

A gas can diffuse into and through a lens material if the molecules of the gas are able to find (and fit through) 'voids' (polymer-free spaces) (Hill, 1978) or 'microvoids' within the structure (Caroline and Ellis, 1986).

An example of this process occurs when a tennis ball is removed from its pressurized storage can. When manufactured the pressure within the can is greater than that inside the ball, the ball will be compressed somewhat and the air trapped inside the ball cannot escape. Over time, the pressure inside and outside the ball will equalize by the pressurized air inside the can *entering* the ball. After opening the can, the air inside the ball *diffuses* through the micropores, or voids, within the rubber shell. In this way the ball eventually loses its bounce and becomes 'flat' (pressure inside the ball will be atmospheric pressure only).

Each material (solid, liquid or gas) has an inherent property called a diffusion coefficient that is given the symbol **D**. It is the D in the equation for oxygen permeability, **P= Dk**. For details of **k** and **P** see the next slide and text.

14

GAS SOLUBILITY

- Sorption process of a gas within the material
- Similar to a sponge which absorbs and holds water
- Gas is dissolved, or solubilized, into the material

98200-10S.PPT

6L298200-10

Gas Solubility

The process of gas solubility in a contact lens can be likened to a sponge that absorbs and holds a liquid (Caroline and Ellis, 1986).

A contact lens material has a specific solubility coefficient that is given the symbol **k** in the oxygen permeability (**P**) equation.

P = Dk

Because of the practical difficulties in measuring k, it is usual to measure the quantity Dk directly rather than by multiplication of its components (D and k) (Fatt, 1976).

III RGP Permeability and Transmissibility

15

RGP PERMEABILITY

- Siloxane bonds (Si-O-Si) provide molecular sized 'voids'
- Size, rotation and flexibility of bonds affect permeability
- Concentration gradient

98300-7S.PPT

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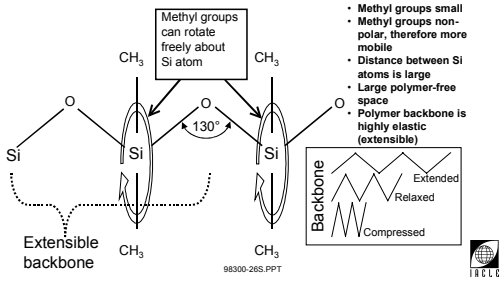
16

RGP MATERIALS
SILOXANE COMPONENT



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17

RGP PERMEABILITY

Three step process:

- Gas dissolves into the anterior lens surface
- Diffusion through the lens proper
- Gas leaves via the posterior lens surface and dissolves into the post-lens tear film

98300-8S.PPT

6L398300-8

18

RGP PERMEABILITY

- Permeability is a function of molecular motion and/or solubility
- Increased permeability with:
  - improved surface characteristics
  - higher temperature
  - decreased cross-linking
  - greater atmospheric pressure
- Polymer compromises

98300-10S.PPT

9L1300-10

Oxygen Permeability of Rigid Gas Permeable Contact Lenses

Most RGP contact lens materials contain siloxane, a generic name for chemical entities involving the silicon to oxygen bond (Si-O-Si), also referred to as the siloxane bond.

Such a bond is capable of rotation and flexure (including significant extension and compression) (slide 16).

During movement, molecular-sized spaces that permit the permeation of gases are formed within the material. Although these spaces exist only momentarily, they are open long enough to enable oxygen molecules to pass (diffuse). They should not be regarded as 'holes'. Rather, they should be viewed as potential voids (Caroline and Ellis, 1986).

The degree to which this process occurs in a material is referred to as the diffusion coefficient (**D**) in the oxygen permeability equation **P = Dk**.

A concentration gradient is formed across the lens thickness, i.e. between the front and back surfaces of the lens *in situ*, due to the consumption of oxygen from the post-lens tear film by the cornea (or cornea and anterior eye in the case of SCLs). Oxygen passes along this gradient, through the lens, towards the eye to supply the anterior cornea via the post-lens tear film.

The diffusion of oxygen through the material is only one component of lens permeability. The value **k** in the permeability equation relates to the solubility of oxygen in the material. Oxygen has a demonstrated preference for dissolving in fluorinated siloxane acrylates rather than siloxane acrylates.

The solubility of oxygen within an RGP material starts with the adsorption of the gas (oxygen) onto the front surface of the lens. The oxygen then dissolves into the lens material through which it passes (diffuses) towards the back surface under the influence of the concentration gradient explained previously. It then leaves the back surface and enters the post-lens or pre-corneal tear film again by a further process of dissolution.

The factors that determine the oxygen permeability of an RGP lens are:

- Polymer composition.
- Temperature.
- Partial pressure at the anterior surface.
- Boundary layers (lens/tear film interface).

Polymer chemists who develop RGP materials must ensure that certain desirable characteristics are not compromised in an attempt to develop a higher level of oxygen permeability. Such characteristics include:

- Rigidity.
- Durability.
- Wettability.
- Long-term stability.

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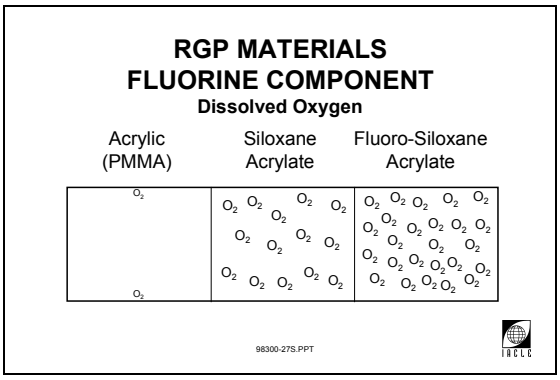

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Permeability of Fluorinated RGP Materials

The siloxane-acrylate RGP contact lens materials were developed further by the addition of a fluorinated monomer. Fluoro-siloxane acrylates owe their higher oxygen permeability to both their fluorine and siloxane components.

However, a siloxane bond provides a much greater level of oxygen permeability than does the addition of fluorine, i.e. all other things being equal, the siloxane factor is more influential than the fluorine one.

While fluorinated lens materials are more oxygen permeable due to the greater solubility of oxygen in them (slide 19), the major benefit of a fluorinated polymer is its improved surface properties compared with those of the siloxane-acrylates. The interaction between the lens and tear film components such as proteins, mucus and lipids is decreased significantly, resulting in fewer deposits.

The combination of enhanced surface characteristics and higher oxygen permeability makes the fluorinated siloxane-acrylates the materials of choice for the majority of patients who require, or elect to use, RGP contact lenses.

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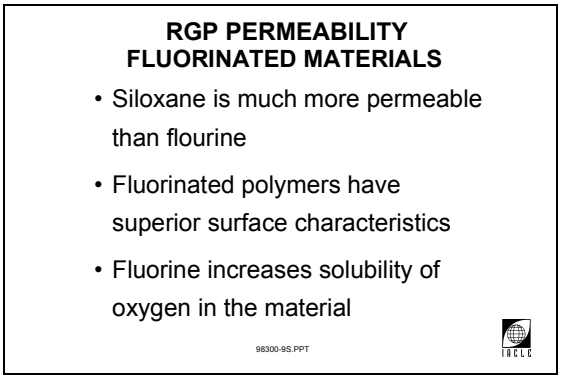

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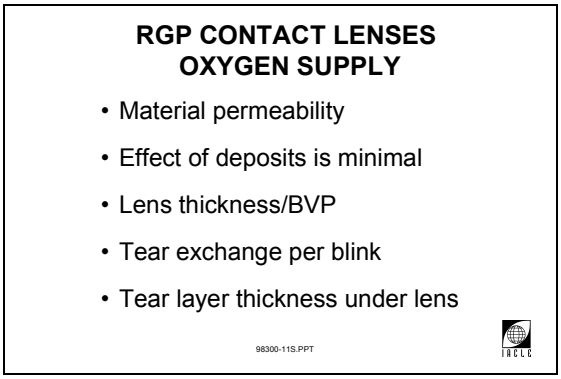

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Corneal Oxygen Supply with RGP Lenses

The supply of oxygen to the cornea during RGP lens wear is determined by a number of factors.

Most oxygen supplied to the cornea diffuses *through* the lens. Therefore, the oxygen permeability of the lens material for an average lens is the prime determinant of oxygen supply to the cornea.

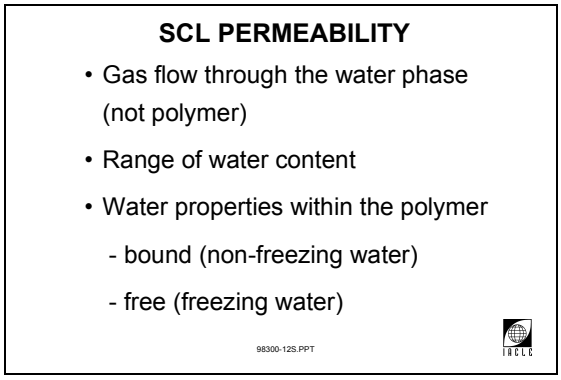
Design characteristics such as lens BVP and average thickness play a significant role in determining the oxygen transmissibility of the lens *in situ.* However, RGP lenses are routinely applied in a relatively narrow range of centre thicknesses. Given this, the material permeability and BVP are of most significance when considering the oxygen performance of a particular lens.

Oxygen is also supplied to the cornea via the tears. The exchange of tear fluid between the tear reservoir at, and under, the lens edge and the tears in the post-lens tear film, results from lens movement on the eye. This is the so-called 'tear pump', the driving force of which is normal blinking.

Despite the much more effective tear pump existing with RGP contact lenses, the tears are a much less effective source of corneal oxygen than transmission *through* highly permeable lenses.

III SCL Permeability and Transmissibility

22



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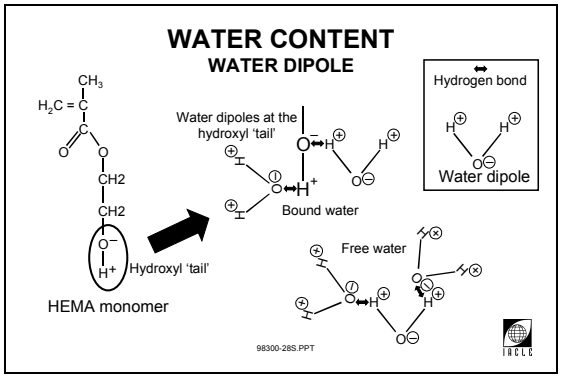

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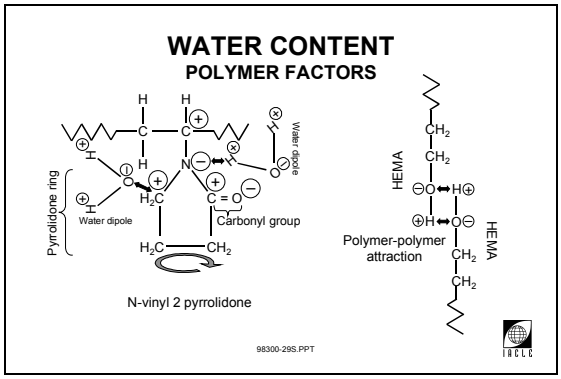

Oxygen Permeability of Hydrogel Materials

Typically, polymers used in the production of hydrogel lenses are characterized by their water content. Usually, the water content is expressed as a percentage of the total lens once it is hydrated fully in normal saline.

The temperature should also be stated because the amount of water contained within the lens is temperature dependent. Usually, as a hydrogel's temperature increases its water content decreases (Tighe, 1989). This has a significant effect on the effective oxygen permeability of the material, as will be seen below.

In addition to temperature, the water content of a material is dictated by the chemistry of the polymer in which it is located. Factors include:

- The type, number and density of charged sites to which the polar water dipole might be attracted (slide 23).
- The existence of other charged species which may compete with water for the same charged sites.
- Polymer-polymer attraction (see right hand side of slide 24).
- The presence of other polymer groups, tails or side-chains competing with water for the same molecular space, or which may shield the charged sites from the water dipole.
- The presence of groups, tails or side-chains that may shield charged sites from other large polar species, which, while too big to gain access, allow the water dipole ready access.

Oxygen flow through a hydrogel contact lens is dependent on the properties of the water 'contained' within the material. Oxygen passes through the lens by virtue of water's inherent permeability to such gases. There is no gaseous phase transfer of oxygen in hydrogels. All transfer occurs in the dissolved phase, i.e. the movement of oxygen through a hydrogel relies on oxygen's solubility in water. (Fatt, 1987). Oxygen molecules do not pass through the material polymer itself, rather they diffuse mostly through the water-filled pores contained within the lens. Some oxygen is dissolved in the material itself.

Therefore, the oxygen permeability of water is the main limiting factor in the amount of oxygen that reaches the cornea via a hydrogel lens. Water is much more permeable to carbon dioxide leaving the cornea largely because of its solubility and chemical interaction with water.

To conceptualize dissolved oxygen, Fatt (1978) uses the analogy of the gas (the identity of the gas is unimportant but it is actually carbon dioxide) present in a sealed aerated (carbonated) beverage. It enters as a gas, is dissolved in the beverage while sealed and exits as a gas once the bottle is opened. Generally, a material's oxygen permeability increases as the water content is increased.

25

SCL PERMEABILITY

- Only free water is available for O_2 transport
- Ratio of bound to free water is influential
- New polymers (siloxane components)
- Effect of tints
  - opaque
  - dyes

98300-13S.PPT

6L398300-13

26

NOVEL SCL POLYMER

- Biphasic block-copolymer
- Siloxane-based polymeric phase
- Coupled with a water phase
- O_2 permeability increases as H_2O content decreases

98300-24S.PPT

6L398300-24

However, the oxygen permeability of water itself imposes an upper limit on the amount of oxygen that can be delivered to the cornea through a hydrogel lens.

Further, the chemistry of water retention within a hydrogel material also determines how easily oxygen can pass through the lens to reach the post-lens tear film. For this reason it is necessary to distinguish between the methods of water retention within the lens matrix.

There are two methods of water retention - bound and free. In a hydrogel material, some of the water, which exists as a charged molecule or dipole, is electrostatically bound to the polymer by the so-called hydrogen bond (slide 23). Its properties are changed by this attraction in such a way that it is no longer available as a vehicle for oxygen transport through the lens. This bound water is also referred to as non-freezing water (Mirejovsky *et al*. 1993).

Other factors are involved in water retention at the molecular level. For example, in the case of vinyl pyrrolidone-containing materials, the main water attractant is the negatively charged nitrogen atom in the bulky and relatively rigid pyrrolidone ring (slide 24).

Although the ring itself can rotate about its connection to the main polymer backbone (i.e. about the C–N bond) its inflexible properties reduce the attraction between itself and adjacent pyrrolidone rings within the material.

Importantly, the protected nature of N allows water to bond while restricting access by other larger entities.

The charges on the carbonyl group (C=O) are small and contribute little to either polymer-polymer or polymer-water attraction and have only limited significance (this section after Frankland, 1973).

Much of the water within a hydrogel polymer is free to carry oxygen from the anterior surface to the cornea. This free water component is also referred to as freezing water. As far as oxygen transport is concerned, the ratio of bound to free water is more important than the total water content of the lens material.

New polymers developed for use as soft contact lenses have the potential to provide significantly improved oxygen supply to the cornea. An example of one such new material is described as a biphasic block-copolymer of a highly permeable siloxane-based polymer, coupled with a water phase. Due to the biphasic nature of this material, the oxygen permeability increases as the water content decreases (Alvord *et al*. 1998).

Tints used in soft contact lenses can affect the oxygen transmissibility. Generally, the transparent dyes have a negligible effect on lens transmissibility. However, opaque tinted lenses can reduce the flow of oxygen to the cornea because the material of the opaque ‘artwork’ is relatively impermeable.

27

SCL TRANSMISSIBILITY

- Thickness considerations:
  - average *vs* centre
  - BVP
- Influences physiological response

98300-14S.PPT

6L398300-14

28

SCL TRANSMISSIBILITY

- Increased Dk/*t* with:
  - higher water content (fixed thickness)
  - thinner lens (fixed water content)
- Highest Dk/*t* with thin, mid-water lenses
- Very heavy deposits can reduce Dk/*t*

98300-15S.PPT

6L398300-15

Oxygen Transmissibility of Hydrogel Materials

The thickness of a hydrogel lens also influences the amount of oxygen transmitted. The thickness characteristics of an SCL influence both the oxygen transmissibility and the clinical performance of the lens.

Calculation of SCL oxygen transmissibility should take into account the *average* thickness of the lens. The centre of the lens may not be representative of much of the lens in terms of the amount of oxygen delivered to the anterior corneal surface. This is particularly so when considering moderate to high minus powers or plus powered lenses.

Thickness also contributes to the dehydration characteristics of the material both on and off the eye. In general, the thicker the lens, the less it will dehydrate. This is due largely to the greater resistance to the bulk flow of water through the lens offered by thicker lenses.

However, thickness also reduces the oxygen transmissibility. If a high water content lens is produced too thin, it causes a higher level of coalescent macro-punctate corneal staining due to pervaporation (Holden *et al*. 1986). Therefore, lens designers and prescribers must balance the desire for high transmissibility (thinner lenses) with preventing pervaporation staining (thicker lenses).

Oxygen transmissibility of a hydrogel lens indicates the expected overall physiological performance of the lens on-eye. Knowing the Dk/*t* of a lens permits the practitioner to classify it on the basis of the Holden and Mertz criteria for safe daily and overnight wear.

Changes in material water content as well as lens design can affect oxygen transmissibility. Based on clinical performance, the practitioner may vary one or both of these variables to achieve higher oxygen supply to the cornea.

For practical purposes, the Dk/*t* of a hydrogel lens is unaffected by mild to moderate deposits on the surface. The oxygen transmissibility may be slightly decreased when very heavy deposits accumulate.

IV Corneal Swelling with Contact Lenses

29

CORNEAL SWELLING
SCL DAILY WEAR

• HEMA material, 8 hours wear

t_c (mm)	Swelling(%)
0.13	8
0.07	5
0.03	1

La Hood, CCLRU Data 98300-16S.PPT

6L398300-16

30

CORNEAL SWELLING
SCL DAILY WEAR

• HWC (75%) material, 8 hrs wear

t_c (mm)	Swelling(%)
0. 3	2
0.15	0.5

La Hood, CCLRU Data 98300-23S.PPT

6L398300-23

31

CORNEAL SWELLING
SCL OVERNIGHT WEAR

Material (8 hrs wear)	Swelling (%)
Low Water	12
Mid Water	10
High Water	11
Novel Polymer	4
Siloxane Elastomer	2.5

La Hood, CCLRU Data 98300-18S.PPT

9L198300-18

Corneal Swelling with Soft Contact Lens Wear

Among the most commonly used hydrogel polymers is hydroxy-ethyl methacrylate (HEMA), the very first successful soft lens material. HEMA is a low water content (approximately 38.0%), non-ionic material which has been used to fabricate lenses using all existing manufacturing technologies.

Interestingly, the method of manufacture influences the physical properties of the lens that results. Oxygen permeability is such a factor and one possible reason for the variation in material Dk reported for HEMA is the failure to note the manufacturing technique used to fabricate the test lenses.

As the thickness of a lens increases, its oxygen transmissibility decreases and a higher level of corneal swelling results. Over an eight-hour period of wear, an ultra-thin HEMA lens may induce only minimal swelling. A thicker lens such as a toric or a plus power (typical t_c = 0.13 mm) induces about 8% corneal swelling.

A 0.15 mm thick lens made from a high water content material (e.g. 74%) results in about 0.5% swelling after eight-hours of daily wear. Such lenses are more suitable for toric and plus prescriptions than are lower water content materials.

In myopic hydrogel extended wear patients, the level of corneal oedema caused during the closed eye phase of wear varies only slightly between lenses that are considered to be optimum for that modality. Such lenses are:

- Ultra thin low water content.
- Thin mid water content.
- High water content.

Typically, these extended wear lenses cause about 8-12% corneal swelling following eight hours of eye closure. This compares very unfavourably with the 3-4% swelling that occurs in the average non-lens wearing eye.

Recent developments in siloxane hydrogel materials for SCL extended wear have produced a significantly better swelling response of approximately 2–3% after eight hours of sleep. In a study by Fonn *et al.* (1998) comparing high Dk (140) siloxane-containing hydrogel lenses (lotrafilcon A) with low Dk (28) hydrogel lenses (etafilcon A), overnight corneal swelling after eight hours sleep was 2.7% (SD ±0.4%) with the high Dk lenses and 8.6% (SD ±0.6%) with the low Dk lenses. The new generation lenses delivering much higher transmissibilities than previously are being used to re-launch soft lens extended wear.

32

CORNEAL SWELLING
RGP DAILY WEAR

Material (8 hrs wear)	Swelling (%)
PMMA	6
Low Dk	3 - 4
Mod Dk	1
High Dk	0

La Hood, CCLRU Data 98300-17S.PPT

6L398300-17

33

CORNEAL SWELLING
RGP OVERNIGHT WEAR

Material (8 h wear)	Swelling (%)
Low Dk	10-13
Mod Dk	7-9
High Dk	5-6

La Hood, CCLRU Data 98300-19S.PPT

6L398300-19

Corneal Swelling with RGP Contact Lens Wear

The higher level of oxygen transmissibility achievable with RGP lenses compared with SCLs results in less corneal swelling during daily lens wear. The additional supply of oxygen via the tear pumping mechanism with RGP lenses, also contributes to the low levels of oedema. Only very low Dk/*t* RGP lenses cause any significant corneal swelling.

When RGP lenses of low oxygen transmissibility are worn overnight, the level of corneal oedema induced is very similar to the best results achievable with current conventional hydrogel lenses.

When highly oxygen transmissible RGP lenses are worn overnight, the 5-6% corneal swelling that occurs compares favourably with the 3-4% in a non-wearer. In extended wear, a major advantage of RGP lenses over SCLs is the rapidity with which the induced corneal oedema resolves following eye opening. This is due primarily to the superior oxygen transmissibility of RGP lenses, as well as the tear exchange that occurs when the lenses begin to move on the cornea once blinking is re-established.

34

SILOXANE ELASTOMER

- A dimethyl polysiloxane polymer
- Inherently hydrophobic
- Pure polymer very oxygen permeable
- Permeability of the 'filled' polymer used is significantly lower

98300-25S.PPT

6L398300-25

35

SILOXANE ELASTOMER
CORNEAL SWELLING

- Less overnight swelling than no lens 2.0% *vs* 3.6%
- Possible reasons:
  - decreased resistance to O_2 flow from lid
  - lens-induced lagophthalmos
  - altered CO_2 level
  - reduced tonicity change

98300-20S.PPT

6L398300-20

Corneal Swelling with Siloxane Elastomer Contact Lens Wear

Siloxane elastomer lenses have the highest oxygen transmissibility of any current contact lenses. They induce zero corneal swelling during daytime wear. The level of swelling following overnight wear is, somewhat paradoxically, less than the non-lens wearing average of 3.6% (Sweeney and Holden, 1987).

A number of possible explanations have been suggested to explain this phenomenon (slide 35) but as yet, the reason remains unclear.

Contact lenses should become even safer in the future because:

- Transmissibilities will improve further as novel materials are developed.
- Pervaporation problems will be reduced allowing the safe use of thinner lenses, thereby offering even greater transmissibilities.
- Surface properties will improve leading to less lens spoilage.
- Our understanding of unresolved microbiological and immunological issues will increase, and appropriate countermeasures will become available.

🕮 References

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Unit 6.4

(2 Hours)

Lecture 6.4: Microbiology and Contact Lens Wear

Course Overview

Lecture 6.4: Microbiology and Contact Lens Wear

I. Introduction to Microbiology

II. Bacteria

III. Other Types of Micro-organisms

IV. Identification of Micro-organisms

V. Ocular Biota and Contact Lens Wear

Lecture 6.4

(2 Hours)

Microbiology and Contact Lens Wear

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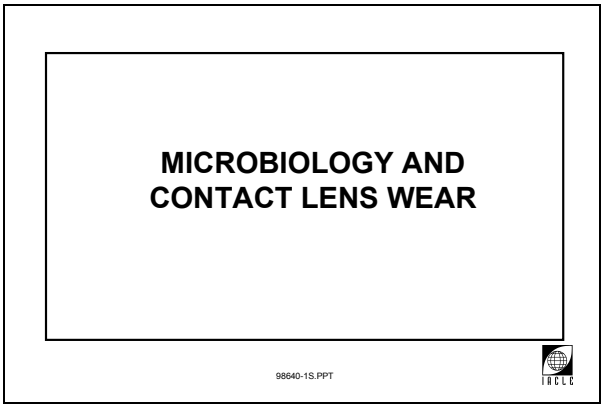
I Introduction to Microbiology

1



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Microbiology and Contact Lens Wear

This lecture:

- Presents an overview of micro-organisms including bacteria (and chlamydiae), fungi, viruses and protozoans.
- Reviews the characteristics of micro-organisms that can cause infection or inflammation in the context of contact lens wear.
- Specifically considers those micro-organisms believed responsible for contact lens-related complications and disease.

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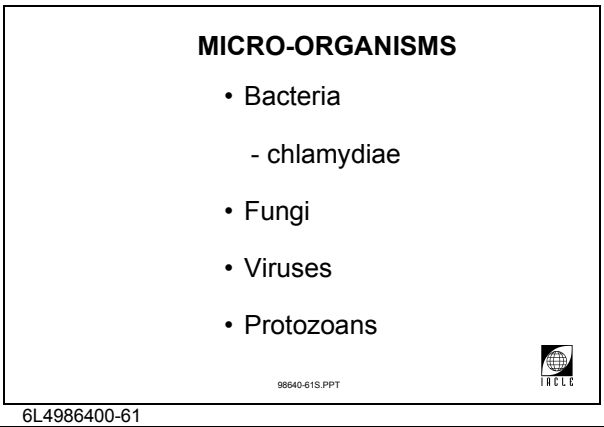

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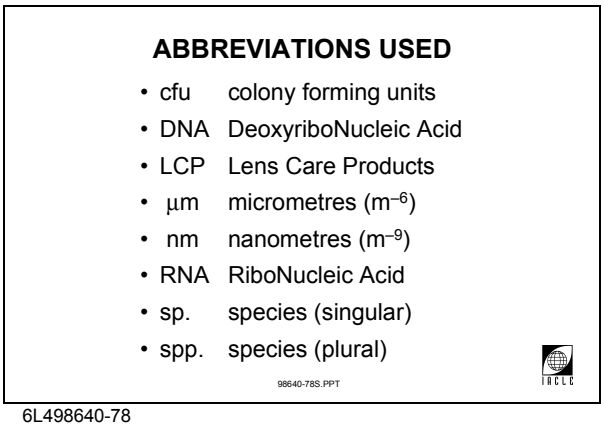

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Abbreviations Used

(Arranged alphabetically)

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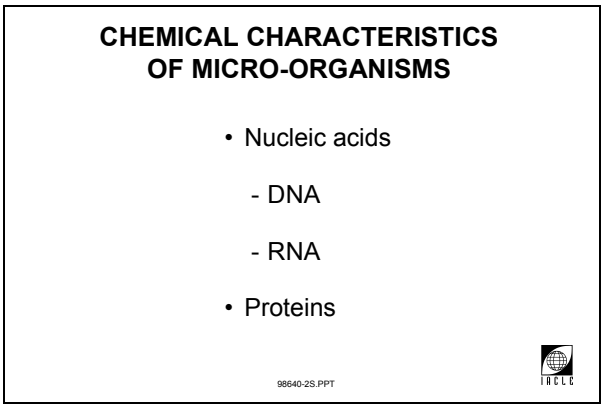

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Chemical Characteristics of Micro-Organisms

Basic chemical components:

- Nucleic acids:
  Nucleic acids, as their name suggests, are located in the nucleus of cells. They code genetic information about a cell, i.e. all the information about the heritable characteristics of the cell.
  They are also involved in protein synthesis that occurs outside the nucleus in the cell's cytoplasm. Cytoplasm constitutes the bulk of the contents of a cell and is mobile, water-rich and gel-like. One definition describes its outer limit as the cell membrane and its inner limit as the nucleus or nuclear body. Another definition describes it as all cell components other than the nucleus but including the other organelles (NIH Website, 2000).
  Nucleic acids may be either DeoxyriboNucleic Acid (DNA) or RiboNucleic Acid (RNA).
  While DNA is metabolically stable once synthesized, RNA is in a dynamic equilibrium with other amino acids present (Ganong, 1979).
- Proteins:
  Proteins are the building blocks of cellular structure. The 'blueprints' of the sequences of amino acids to be synthesized into proteins are carried to the synthesis sites by RNA. The proteins formed include all the enzymes that in turn control cell metabolism. Enzymes therefore, are synthesized proteins that determine metabolic activity.

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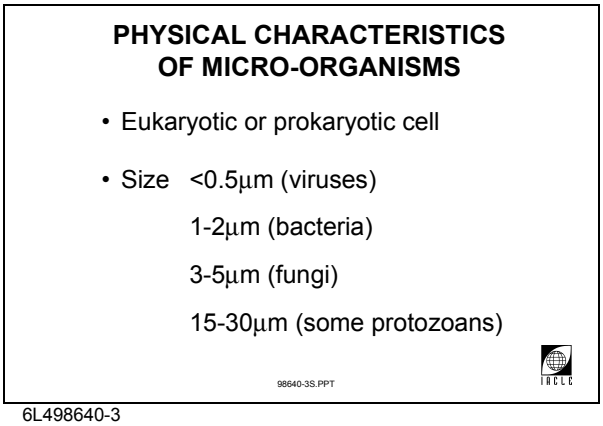

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Physical Characteristics of Micro-Organisms

The structural unit of fungi is a eukaryotic cell, i.e. one that has a nuclear membrane. The cells have multiple chromosomes, a nuclear membrane and membrane-bound organelles. They divide by mitosis, a process in which the chromosomes duplicate themselves and then divide in such a way that each daughter cell has a full complement of chromosomes (Ganong, 1979).

The structural unit of bacteria is the less complex prokaryotic cell. This cell type has a single chromosome, no nuclear membrane or membrane-bound organelles and divides by binary fission, i.e. first the nuclear material and then the cytoplasm divide into two equal parts. This division process is also common to protozoans.

Viruses are acellular, non-living, but biologically formed supramolecular assemblies, i.e. they consist of macromolecules. They consist of a nucleic acid molecule surrounded by a protective shell of protein molecules call a capsid (after Lehninger, 1982). The virus life-cycle is detailed later.

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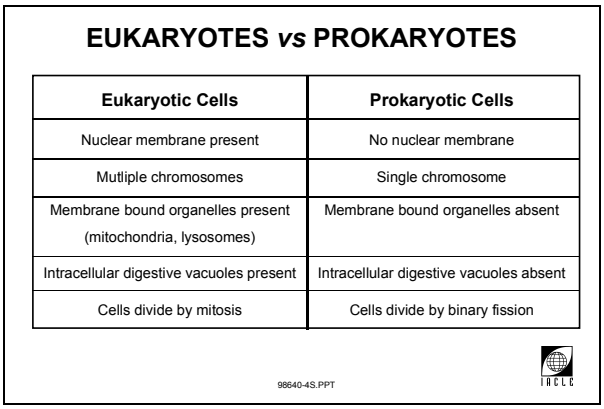

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Eukaryotes *versus* Procaryotes

Eukaryotic cells are more complex than prokaryotes. The table in slide 6 summarizes the differences between the two cell types.

II Bacteria

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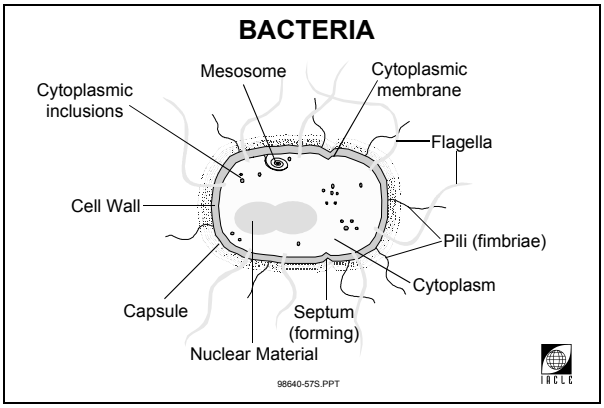

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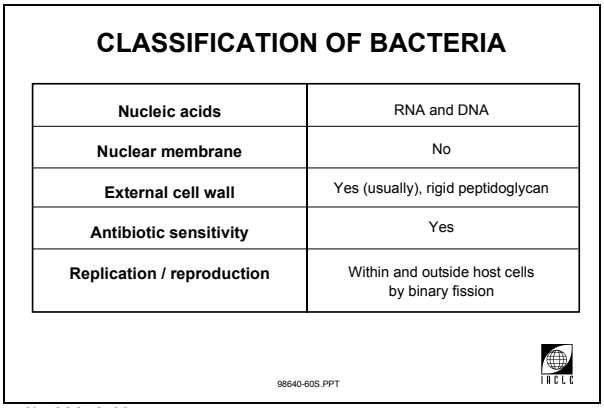

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Bacteria: Structure and Classification

A representation of the structure of generalized bacteria is shown in slide 7. Bacteria can be classified by:

- Morphology.
- Staining characteristics.
- Growth requirements.
- Biochemical structure.
- Antigenic structure.

On the basis of these factors, bacteria have been classified into:

- Orders.
- Families.
- Genera.
- Species.

Within a species, bacteria differing from each other in minor respects are variously designated groups, types or varieties.

As far as possible, each distinct kind of bacterium is assigned a name indicating its genus and species.

The generic name is often abbreviated according to a convention. Thus we have *Staphylococcus aureus* (*Staph. aureus*) or *Mycobacterium tuberculosis* (*Myco. tuberculosis*).

Slide 8 summarizes some of the classification criteria for bacteria.

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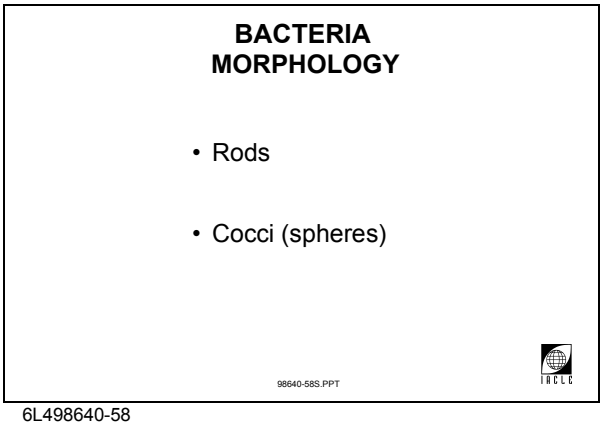

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Morphology of Bacteria

Normally, bacteria take the form of either a rod or a sphere (cocci).

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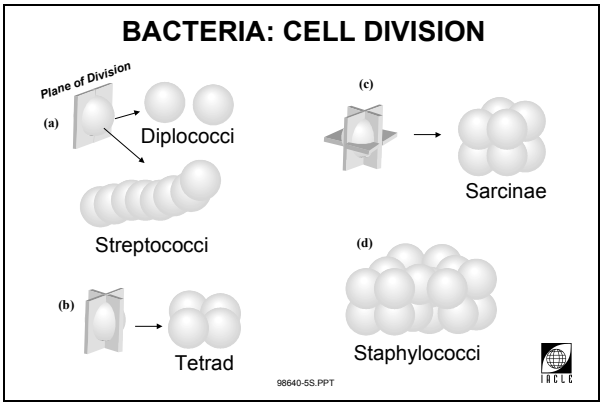

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Morphology of Spherical Bacteria

The actual morphology of groups of cells depends on how single cells divide. Some possible patterns are shown in slide 10.

For spherical bacteria (called cocci) the cell division can occur in the following ways:

(a) Division in one plane – either a pair called diplococci (e.g. *Neisseria* spp.) or a chain (e.g. *Streptococcus* spp.).
(b) Division in two planes – group of four (tetrads, e.g. *Micrococcus* spp.).
(c) Division in three planes – group of eight either cube (sarcinae), or cluster (e.g. *Staphylococcus* spp.).

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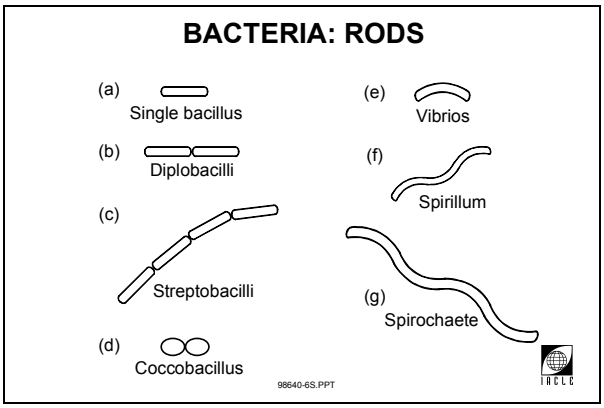

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Bacteria: Rods

The morphology of non-spherical bacteria is described according to the following:

- Filamentous rods – elongated forms.
- Coccobacilli – short, rounded rods (e.g. *Acinetobacter* spp.).
- Vibrios – comma-shaped rods (e.g. *Vibrio cholerae*).
- Spirilla – spiralled, inflexible rods.
- Spirochaetes – very thin, spiraliform, flexible filaments.

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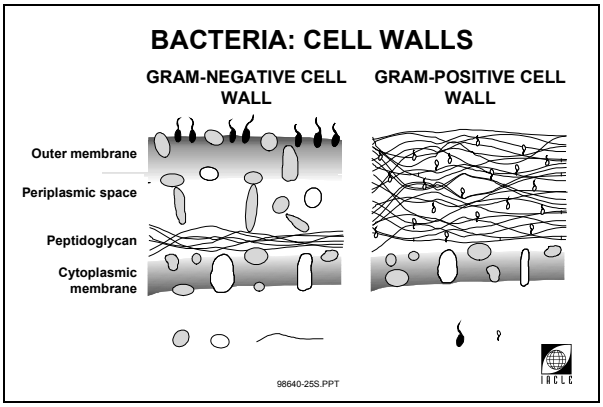

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Bacteria: Cell Walls

The cell wall accounts for up to 20% of the total dry weight of the cell and much of the cell's metabolic capabilities are devoted to its manufacture. Its main function is a mechanical one, enabling the delicate cytoplasmic membrane to withstand the high internal osmotic pressure found in bacteria.

Bacteria can be classified by the reaction of their cytoplasm and cell wall structure to the Gram stain. This technique mixes a violet dye (crystal violet) and iodine that combines with the cytoplasm. The Gram-positive cells retain the stain when challenged with acetone and remain purple. Gram-negative cells lose the purple stain, de-colourize and take up a red counter stain. Examination of the Gram stains also allows the shape of the cells to be determined.

Gram-positive organisms:

- Have a thick peptidoglycan layer (a mucopeptide of alternating N-acetyl muraminic acid and N-acetyl glucosamine) cross-linked with peptide sub-units that give rigidity to the structure, within which teichoic or teichuronic acid is interspersed.

Gram-negative organisms:

- A smaller peptidoglycan layer that is surrounded by an outer protein membrane and lipopolysaccharide, enclosing the periplasmic space.

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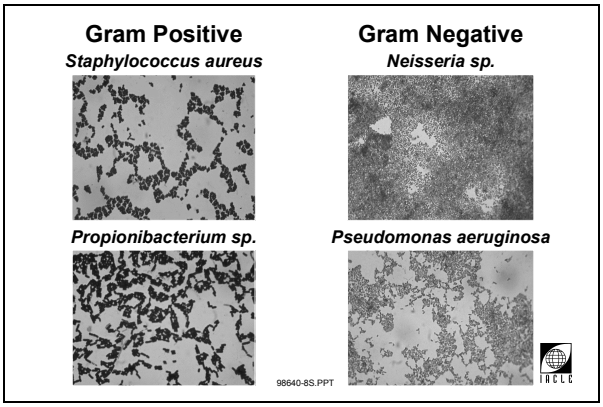

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Bacteria Associated with Eye Infections

The following are examples of Gram-positive and negative rods and cocci that can colonize or cause disease in the eye (slide 13):

- Gram-positive coccus: *Staphylococcus aureus* (infection at all ocular sites).
- Gram-negative coccus: *Neisseria* spp. (conjunctivitis in children).
- Gram-positive rod: *Propionibacterium* spp. (colonizes fornices, rarely causes infection, reported in dacrocystitis).
- Gram-negative rod: *Pseudomonas aeruginosa* (causes mainly contact lens-associated infections).

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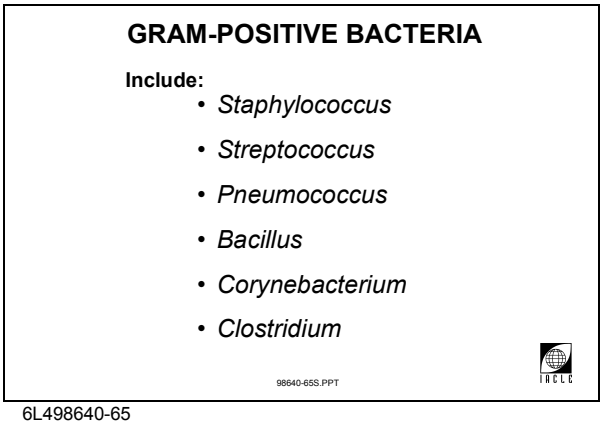

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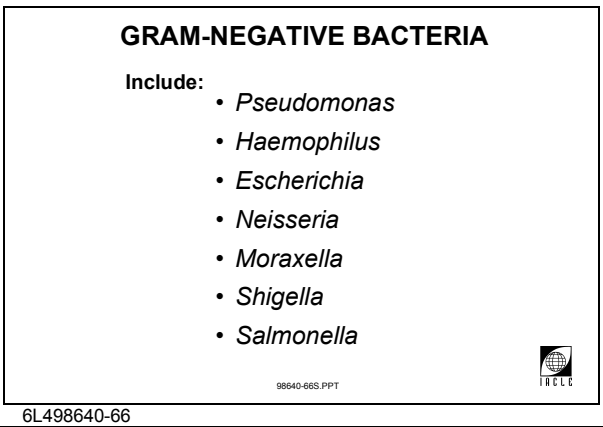

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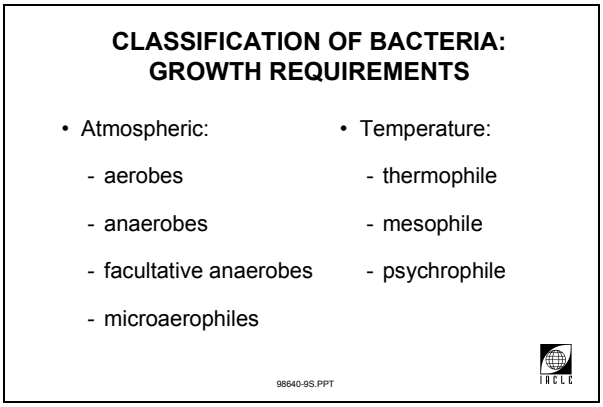

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Classification of Bacteria: Growth Requirements

Atmospheric:

- Aerobes – require O_2 for growth.
- Anaerobes – grow only in the absence of O_2.
- Facultative anaerobes – grow either with or without O_2.
- Microaerophiles – grow in trace O_2 and CO_2.

Temperature:

- Thermophile – grow at 55-80^{o} C.
- Mesophile – grow at 25-40^{o} C.
- Psychrophile – grow at <20^{o} C.

Most ocular pathogens are aerobic/facultative anaerobes that are mesophilic.

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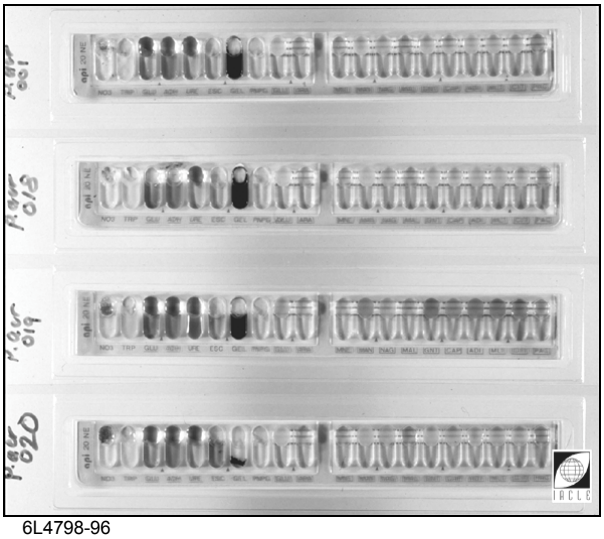

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Classification of Bacteria: Biochemical

Bacteria may be classified according to their biochemical or metabolic reactions. These include:

- Sugar metabolism.
- Protein metabolism.
- Enzyme production.
- Other biochemical reactions.

Commercially available kits allow a wide range of miniaturized biochemical tests to be set up simultaneously on one isolate (a pure sample of bacterial suspension).

After incubation, the results are scored as positive or negative and the scores compared with a database that gives a 'best fit' identification for the isolate.

Slide 17 shows fermentation of different sugars by strains of *Pseudomonas aeruginosa* using an api™ test kit.

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CLASSIFICATION OF BACTERIA: ANTIGENIC

Serotyping, antigenic determinants:

- Envelope
- Flagellae
- Capsule

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Classification of Bacteria: Antigenic

Serotyping is used to differentiate bacteria based on the presence of flagellar, capsular or envelope antigenic determinants and their reaction with specific antisera.

It is particularly useful in delineating species such as *Pseudomonas aeruginosa* and *Legionella pneumophila.*

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BACTERIA AND DISEASE: DEFINITIONS

- Infection
- Colonization
- Asymptomatic carriage
- Virulence or pathogenicity

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Bacteria and Disease: Definitions

- **Infection**. A bacterium capable of causing disease is established in the body.
- **Colonization**. Organisms not capable of causing disease can persist in the body.
- **Asymptomatic Carriage**. Infection by an organism without symptoms of disease.
- **Virulence or Pathogenicity**. The ability of a bacteria to cause infection.

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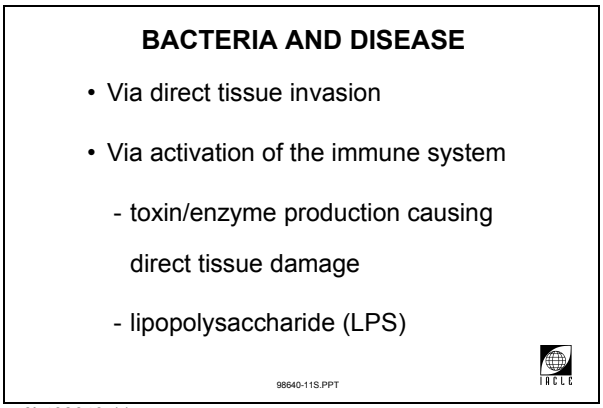

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Bacteria and Disease

Bacteria may cause disease either by:

- Tissue invasion: replication of micro-organisms, tissue destruction and activation of defence mechanisms.
- Immune system activation:
  - toxin or enzyme production can cause tissue damage and can activate the immune system indirectly
  - endotoxin or lipopolysaccharide (LPS) is an outer membrane component of Gram-negative bacteria, which can cause antibody production by B cells, cytokine production by a range of host cells, neutrophil migration and complement activation. For more details of this, and related matters, refer to Lecture 6.5.

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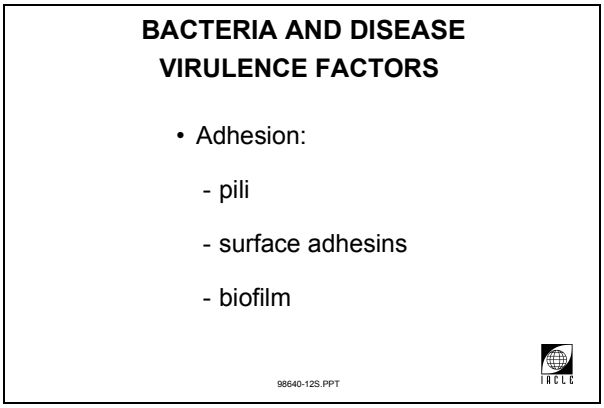

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Bacteria and Disease: Virulence Factors

Virulence factors are the traits that enable bacteria to cause disease. These are:

- **Adhesion:** Adhesion to host cells is an important virulence factor. It is necessary for colonization of a tissue's surface. Adhesion mechanisms include:
  - pili: long protein rods that extend from the bacterial surface and which can bind to carbohydrate moieties on host cell walls
  - adhesins: bacterial surface proteins that can also mediate adhesion between bacteria and host cells
  - biofilm formation: a film in which bacteria are held together by a polysaccharide matrix. These are clinically important for patients who have catheters or plastic implants.

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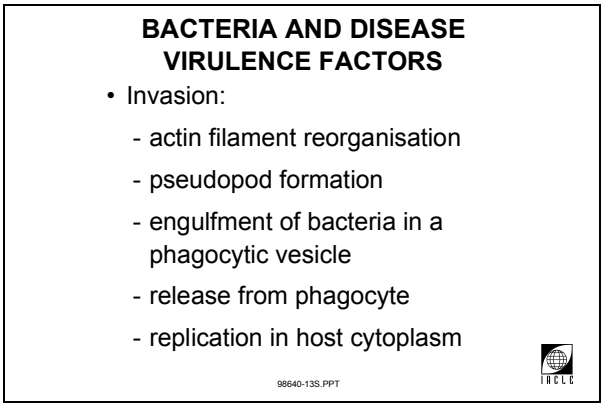

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- **Invasion:** Bacterial invasins are proteins that cause rearrangement of actin (thin contractile filaments) in normally non-phagocytic host cells, i.e. cells that do not ingest foreign particles, other particles or cells that are harmful to the parent organism. Bacteria are ingested into the cells by engulfment in a phagocytic vesicle. Once inside the cytoplasm, bacteria are released from the phagosome and replicate within the host cell's cytoplasm.

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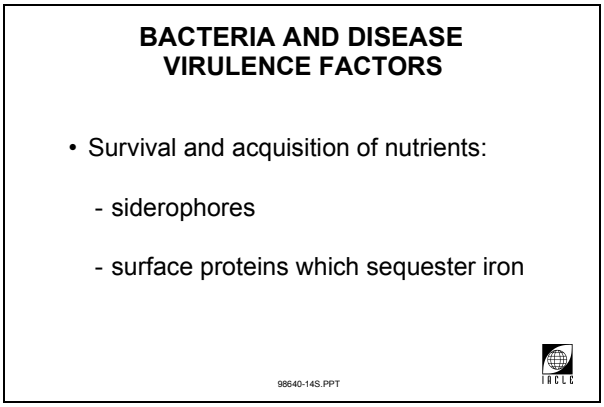

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- **Survival and acquisition of nutrients.** The environment within tissues is generally low in nutrients. To survive, bacteria must sequester nutrients. One approach to compensating for the low iron concentration in the environment is the production of siderophores. Alternatively, surface proteins may remove iron from host iron-binding proteins like lactoferrin or transferrin.

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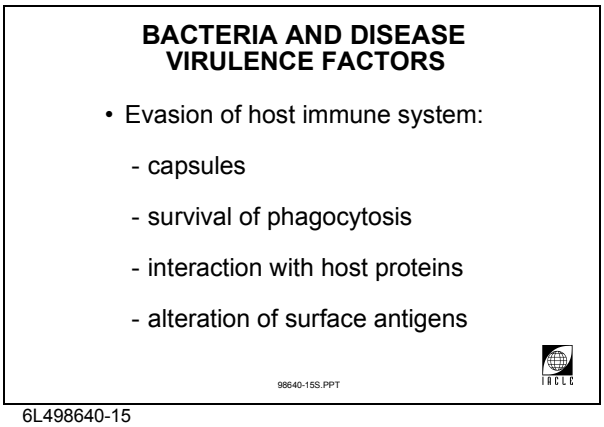

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- **Evasion of the host immune system.** Caspules minimize complement activation and prevent ingestion of bacteria by phagocytes. Some bacteria can survive phagocytosis by:
  - resisting defensins
  - detoxifying reactive oxygen species
  - reducing the strength of a respiratory burst by host cells
  - resisting lysozomal proteases.

  Some bacteria use host proteins like fibronectin as a coating to avoid detection by the immune system. They may also alter their surface antigens to achieve the same effect.

III Other Types of Micro-Organisms

III.A Chlamydiae

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CHLAMYDIAE

- Small intracellular parasite (recognized as bacterial species)
- When Gram stained - appear as tiny (0.2-1μm) Gram-negative bacteria
- Able to synthesize protein, DNA & RNA

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Description of Chlamydiae

This species of organism is an intracellular parasite that was originally classified as a virus. It is now recognized as a bacterial species, but it shares some features with viruses.

Chlamydiae are small in size (0.2-1μm) with an outer membrane similar to Gram-negative bacteria.

Unlike viruses, Chlamydiae can make their own DNA, RNA and protein. However, they rely on host cells for energy sources like adenosine triphosphate (ATP).

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CHLAMYDIAE LIFE CYCLE

- Elementary Body (EB) internalized by host cells
- Organism survives in phagosome, and EB turns into Reticulate Body (RB)
- RB is an intracellular, non-infectious replicating form
- Phagosome enlarges forming an Inclusion Body (IB)
- Some RBs transform (revert) into EB form
- Host cell releases EBs on lysis

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Life Cycle of Chlamydiae

The extracellular form of chlamydiae is called an elementary body (or EB). The EB enters a host cell such as an epithelial cell and is internalized in a phagosome but not destroyed.

The EB can transform itself into an intracellular form of the organism that is capable of replication. This form is called the reticulate body (RB). The phagosome gradually enlarges forming an inclusion body (IB).

Some RBs transform back into the infectious form of the cell (EB), i.e. they revert to their previous form. When the host cell lyses (disintegrates), infectious EBs are released.

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CHLAMYDIAE AND OCULAR DISEASE

- Trachoma
- Inclusion conjunctivitis
- Diagnosis is difficult

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Chlamydiae and Ocular Disease

In the eye, chlamydiae cause trachoma and the sexually transmitted disease inclusion conjunctivitis.

The diagnosis is difficult, since a positive culture is based on the presence of an inclusion body in tissue culture cells. Chlamydiae, like viruses, do not grow on conventional laboratory media such as blood agar.

III.B Fungi

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FUNGI

Include:

- *Candida albicans*
- *Aspergillus*
- *Fusarium*
- *Histoplasma*
- *Trichophyton*

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Fungi: Forms

Most fungi exist in soil, where they degrade organic matter. They are either aerobic, or facultative anaerobic eukaryotes that can exist in three forms.

- Unicellular – this form reproduces by budding, e.g. yeasts such as *Candida* spp. Typically round or oval cells, 4-25μm in diameter.
- Multicellular – this form is composed of filaments (hyphae), e.g. moulds such as *Aspergillus* spp. Hyphae can be 5-50μm in length and can interweave to form a mycelium.
- Dimorphic – this form can be uni, or multi-cellular, depending on environmental conditions.

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FUNGI: FORMS

- Eukaryotes
- Unicellular - reproduce by budding
- Multicellular - composed of filaments (Hyphae)
- Dimorphic - can be uni or multi-cellular

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FUNGI: CLASSIFICATION

Based on spore type and mode of spore production. Types include:

- Zygomycetes/Phycomycetes
- Ascomycetes
- Basidomycetes
- Deuteromyctes/Fungi Imperfecta

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Fungi: Classification

Classification of fungi is based on spore type and mode of spore production (asexual or sexual). There are four groups, all of which include both yeasts and moulds.

- **Zygomycetes/Phycomycetes** – asexual (sporangiospores) or sexual (zygospores) spore production. Spores are formed in a walled sporangium**.** Examples include *Rhizopus* spp. and *Mucor* spp. This group can cause opportunistic disease in humans.
- **Ascomycetes** – this group has septa within the mycelia. They can reproduce sexually (ascospores) or asexually (conidia) and do not produce disease in humans. An example of this group is *Saccharomyces* spp. This fungus causes alcohol fermentation.
- **Basidomycetes** – this group can reproduce asexually (conidia) or sexually (basidiospores) and rarely causes human disease. This group includes mushrooms.
- **Deuteromyctes/Fungi Imperfecta** – this group can not reproduce sexually. The spores are called conidia. The major human pathogens from this group include *Aspergillus* spp., *Fusarium* spp., *Alternaria* spp., *Phoma* spp. and *Penicillium* spp.

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CLASSIFICATION OF FUNGI

Nucleic acids	RNA and DNA
Nuclear membrane	Yes
External cell wall	Yes, rigid chitin
Antibiotic sensitivity	No
Replication / reproduction	Within and outside host cells by binary fission and sexually

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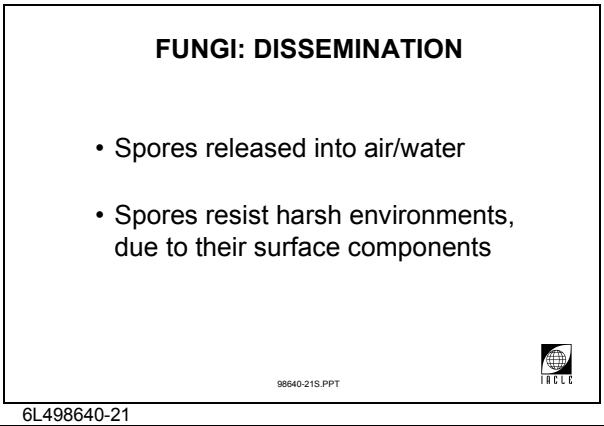

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Fungi: Dissemination

Fungi release spores into the environment. These are then distributed via air and water.

The spores are highly resistant to extremes of temperature and pH. This resistance is partly due to their surface components such as hydrophobic glycoproteins or lipo-glycoproteins.

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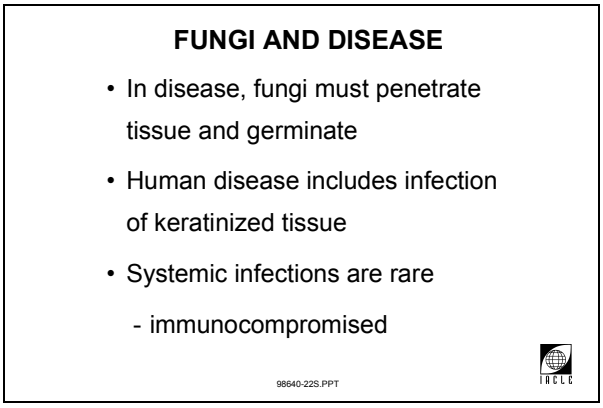

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Fungi and Disease

While fungi are ubiquitous in the environment and frequently found on the skin, they rarely cause infection. To produce disease, fungi must penetrate the tissue and germinate.

Human fungal infections include tinea, ringworm due to *Trichophyton* spp., and *Microsporum* spp.

Systemic infections are rare due to inhibitory factors in tissue and serum.

Fungi may cause indolent (slow-healing) chronic infections particularly in immuno-compromised individuals.

Yeasts are a major cause of infection in patients undergoing chemotherapy, in AIDS and organ transplant patients and in long-term corticosteroid users.

An example of a commensal organism producing disease is *Candida* spp. that causes thrush. *Candida* can also cause endocarditis or fungaemia due to colonization of indwelling catheters or heart valves.

III.C Viruses

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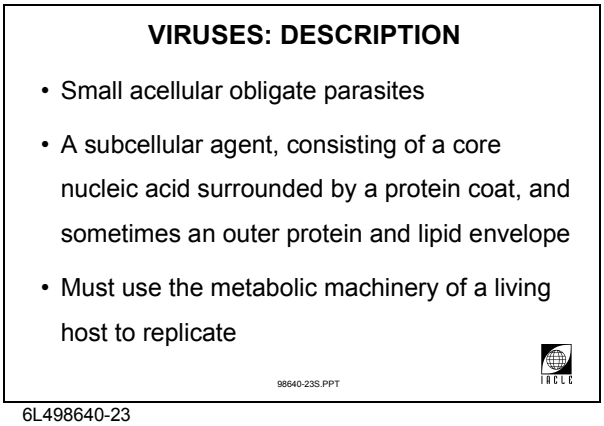

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Viruses: Description and Classification

Viruses are small (20-250 nm), acellular obligate parasites which differ from cellular organisms such as bacteria in their structural and chemical composition and mode of growth. Obligate parasitic organisms require a host for survival and are unable to survive for long periods external to a host.

Viruses are responsible for many human, animal, plant and microbial diseases.

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VIRUSES: CLASSIFICATION

Nucleic acids	RNA or DNA
Nuclear membrane	No
External cell wall	No
Antibiotic sensitivity	No
Replication / reproduction	Within host cells

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VIRUSES: LIFE CYCLE

- Extracellular form = virion
  - double/single stranded DNA or RNA molecule (nucleocapsid) within a protein coat or capsid
- Intracellular form
- Differentiation based on nucleic acid packaging

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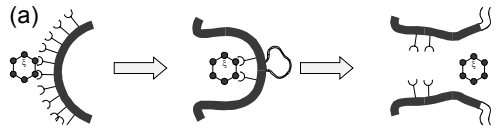
Viruses: Life Cycle

Viruses can exist in either an intra or extracellular form. When outside the cell that formed it, a virus is a non-living particle called a virion. A virion consists of either a single or double stranded DNA or RNA molecule (nucleocapsid) within a protein coat (capsid). Virions contain no mechanism for growth or multiplication and therefore require a host cell. Once a viral particle, or its nucleic acid component, gains entry into its specific host cell, it behaves like an intracellular parasite. The viral nucleic acid carries the genetic message for specifying the entire structure of the intact virion (after Lehninger, 1982).

Virus differentiation is based on how the nucleic acid is packaged. It may be rod shaped, helical, spherical or isometric.

37

VIRUS INVASION



Adsorption of enveloped or non-enveloped virus to cell membrane receptors

Invagination of membrane to form vesicle. Vesicle fuses with lysosome

Lysosomal-vesicle fusion causes release of nucleocapsid. Enveloped virus has envelope digested by lysosomal enzyme

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Viruses and Disease

Viruses are specific to animal, plant or bacterial cells (*bacteriophages*), and probably invade using a range of mechanisms. These include:

- Binding to glycoprotein cell surface receptors causing invagination of the cell membrane, and vesicle formation (*endocytosis*). Once vesicles fuse with lysozomes, enzymes digest the viral envelope and the capsid is released into the host cytoplasm (slide 37).
- Fusing with the cytoplasmic membrane so that the nucleocapsid passes directly into the cytoplasm (slide 38 (b)).
- Directly penetrating the cytoplasmic membrane. Small viruses penetrate the cell wall and shed the capsid coating (slide 38 (c)).

In the intracellular phase the capsid disappears. The host cell then replicates the genetic material and the specific capsid proteins of the virus.
To spread an infection, viruses must be released from the host cell after replication. Sometimes viruses without envelopes are released from the host cell only when the cell has been killed.
Where viruses have a protein/lipid envelope, the mechanism of release is more complex. In such cases, the virus uses, and reorganizes, the part of the host cell membrane that contains specific viral

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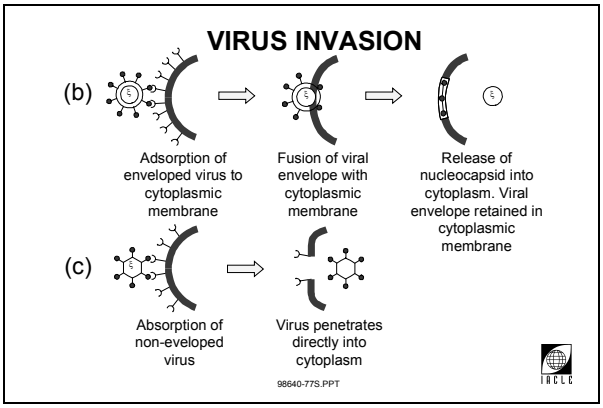

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cell receptors. This is similar to the process of endocytosis in which the virus is taken up by the host cell.

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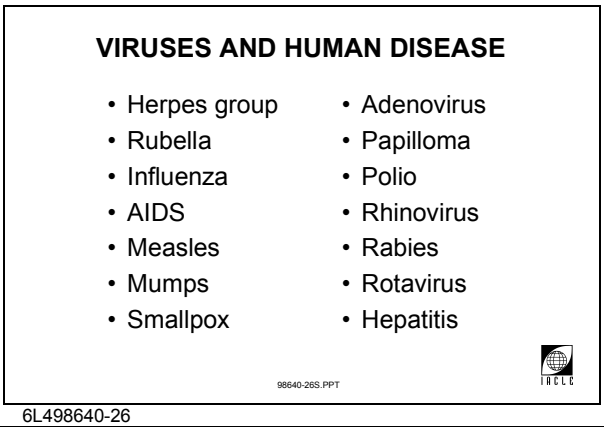

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Viruses and Human Disease

Herpes viruses are the most common cause of viral eye infections, followed by adenoviruses.

The Herpes group includes cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, herpes simplex virus, and human herpesvirus-6.

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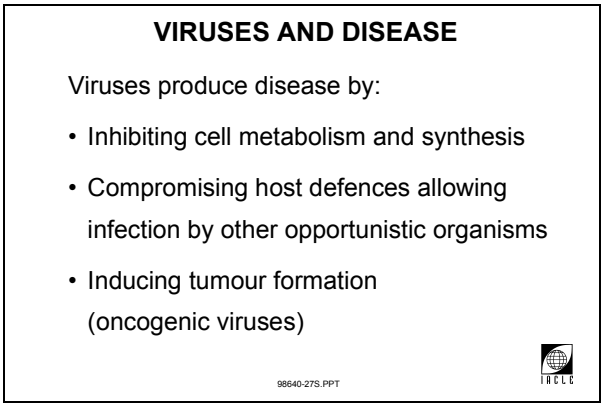

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Viruses and the Disease Process

Viruses can produce disease in several ways. These include:

- Directly, by inhibiting cell metabolism and synthesis. Cells infected by viruses lyse, which can lead to temporary or permanent loss in cell function.
- Indirectly, by compromising host defences, so that colonization by opportunistic organisms such as bacteria can occur. An example of this is the influenza virus that damages the respiratory epithelium and cilia. Once damaged, the surface cannot be cleared of bacteria. Bacteria such as *Haemophilus influenzae* are then able to adhere to the damaged tissue, colonize and produce disease.
- By inducing tumour formation (*oncogenic* viruses).

41

ONCOGENIC VIRUSES AND DISEASE

Oncogenic viruses transform cells in the following ways:

- Viral nucleic acid becomes associated with cell genome, so cell does not lyse
- Transformed cells show altered morphology, metabolism, growth patterns and chromosomal abnormalities
- Cells produce tumours in animal models

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Oncogenic Viruses and Disease

Both DNA and RNA viruses carry genes closely related to host cell genes. These genes are able to transform cells and alter their physiological properties. Some of these changes include:

- The viral nucleic acid becomes associated with the cell genome and the infected cell does not lyse.
- Transformed cells become rounder, lose their orientation pattern in cell culture and show altered metabolism, growth patterns and chromosomal abnormalities.
- Cells can produce tumours if injected into an animal model.

It seems that oncogenic viruses are only able to produce tumours in conjunction with other factors and are probably not the sole cause.

III.D Protozoans

42

PROTOZOANS

- Unicellular
- Parasitic or free living
- Often dual life cycle
- Few cause human disease

98640-29S.PPT

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Protozoans

Protozoans are a diverse group of unicellular organisms, ranging from 5μm – 1mm in diameter. Many are aquatic and they may live either independently or as parasites.

A dual life cycle means that many protozoans can encyst in adverse environmental conditions and survive outside a host for extended periods.

Of the 40,000 species of protozoans, only a few can cause disease in humans.

43

PROTOZOANS: CLASSIFICATION

- Magistophora
- Sarcodina/Rhizopodia
- Sporozoa
- Ciliata

98640-30S.PPT

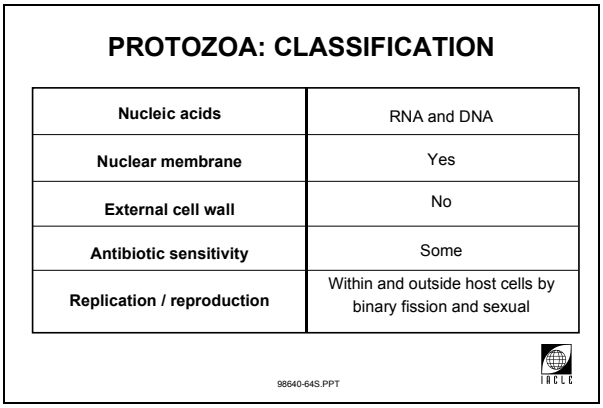
6L498640-30

Protozoans: Classification

Protozoans are divided into four groups, based on their motility. Each group contains isolates capable of producing human disease.

- **Magistophora** – Use whip-like flagella to move. Examples of this group include *Giardia* spp., *Trichomonas* spp. and *Trypansoma* spp.
- **Sarcodina/Rhizopodia** – Amoeboid protozoans which move by extending pseudopodia (literally, false feet). Examples include *Acanthamoeba* spp., *Naegleria* spp. and *Entamoeba* spp.
- **Sporozoa** – This group has no movement organelles. Examples of this group include *Cryptospordium* spp., *Plasmodium* spp. and *Toxoplasma* spp.
- **Ciliata** – Use cilia on the cell surface to move. An example is *Balantidium* spp.

44



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IV Identification of Micro-Organisms

45

MICRO-ORGANISMS: IDENTIFICATION

- Presence of micro-organisms in disease
- Assists in choice of therapeutic agent
- Collection techniques
- Type of specimen

98640-68S.PPT

6L498640-68

Identifying Micro-Organisms

The principal roles of a microbiological laboratory are to determine whether micro-organisms are involved in a disease process and if so, to identify those organisms. Correct identification permits the clinician to initiate the most effective treatment regimen. An initial treatment regimen may be altered should subsequent laboratory testing indicate that a more efficacious therapeutic agent is available.

The method by which a specimen is collected from the patient and the type of specimen taken are key factors in allowing the microbiologist to correctly identify the disease-causing organism.

46

SPECIMENS FOR CULTURE

- Swabs
- Fluids
  - exudate
  - excreta
- Tissue
- Volume
- Transport to lab

98640-69S.PPT

6L498640-69

Specimens for Culture

Numerous techniques and tools are available to collect specimens for laboratory analysis (slide 47).

The type of specimen, how it is collected, its quality and quantity all contribute to the accuracy of identification of any micro-organism.

47

6L40181-98

48

MICROBIOLOGICAL TESTING

- Macroscopic observations
- Microscopic examination
- Cultures
- Antigen detection or gene sequence

98640-70S.PPT

6L498640-70

Laboratory Testing Techniques

The microbiologist determines the most effective method for analyzing the specimen sent to the laboratory. A basic analysis of the specimen by simple observation is typically done when the specimen arrives for examination.

More detailed assessment of a specimen is achieved by microscopic examination utilizing either light or electron techniques.

The cultivation of micro-organisms from a specimen is the mainstay of laboratory investigation. This technique is very labour-intensive and days to weeks may be required before a conclusive assessment can be made.

Non-culture techniques do not require the multiplication of the micro-organism before it can be identified, i.e. the organisms do not need to be viable. The detection of microbial antigens in specimens can provide results within hours. Other non-culture methods, such as the use of DNA probes and amplification of DNA by the polymerase chain reaction, may require up to 1-2 days to finalize.

49

MICROBIOLOGICAL FINDINGS

- Require interpretation
  - type of organism
  - patient's condition
  - therapy optimised

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Microbiological Findings

Results from laboratory tests must be interpreted with skill and precision by the microbiologist as they will play a significant role in choosing the therapeutic agents to be used in treating the patient's condition.

50

EXAMINATION OF CULTURES

- Rapid tests
- Incubation
  - short-term
  - prolonged
- Antibiotic susceptibility tests

98640-71S.PPT

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Examination of Cultures

In some cases, assessment of a culture is possible after 18 hours of incubation when colonies become visible. Identification of some micro-organisms such as fungi requires a much longer incubation period.

Once the organism is identified, antibiotic susceptibility testing may be carried out to establish the appropriate therapy.

51

CULTURE OF MICRO-ORGANISMS

- Solid nutrient media
  - blood agar
  - chocolate agar
- Liquid media
  - thioglycollate broth

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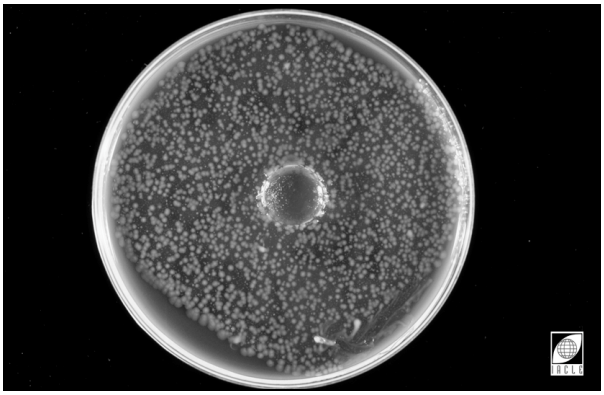

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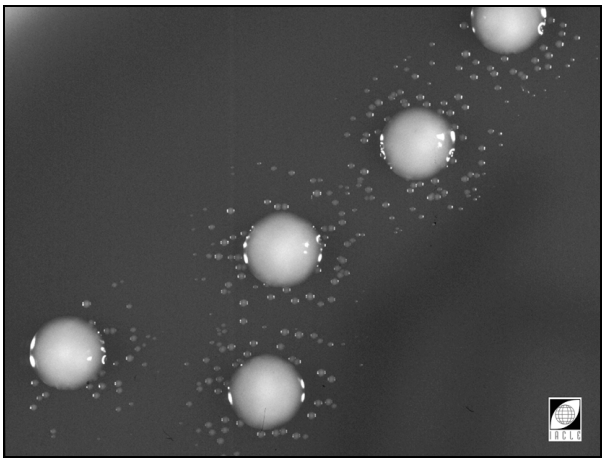

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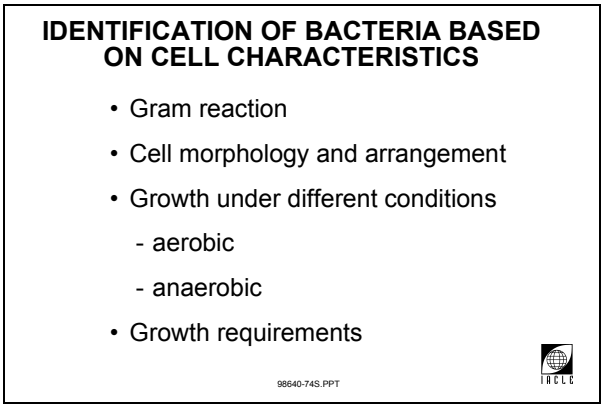
Culture of Micro-Organisms

Animal blood (sheep or horse) is used to produce an enriched (nutrient) solid media that is capable of growing bacteria and fungi on its surface (slides 52 and 53). This is described as blood agar. If the blood is heated to 60°C before it is added to the agar medium, it takes on the appearance of chocolate, hence the name 'chocolate agar'. The agar medium itself is usually a gelatinous product of algae (GardenWeb Website, 2000).

The colonies of different species often have characteristic appearances that can aid their identification.

Cultures can also be made in liquid media (broth) and growth detected by assessing the development of turbidity. This technique does not permit the observer to tell if there is more than one species present in a liquid culture, nor whether there are few or many organisms.

54



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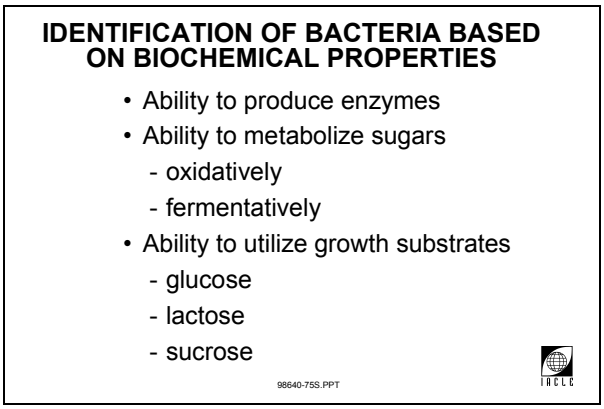
Identifying Bacteria Based on Cell Characteristics

Cell characteristics enable the microbiologist to identify the types of bacteria associated with disease processes. Gram staining is most important for studying bacteria. It enables the classification of bacteria as either Gram-positive or Gram-negative and allows the shape of the cells to be noted.

Further classification is made based upon the ability of the cells to grow under either aerobic or anaerobic conditions. Simple or fastidious growth requirements are also noted when attempting to identify the bacteria.

55



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Identifying Bacteria Based on Biochemical Properties

Further identification of bacteria that have been isolated in pure cultures is often made on the basis of their biochemical properties. For example, the production of the enzyme coagulase distinguishes *Staph. aureus* from *Staph. epidermidis*.

Some species can utilize sugars such as glucose and produce acid by both aerobic (oxidative) and anaerobic (fermentative) pathways.

Tests using a range of substrates for growth can be performed individually in broth media containing the test sugar.

V Ocular Biota and Contact Lens Wear

56

EXTERNAL OCULAR BIOTA

- Sparse colonization of ocular surface
  - lids 60% of cases
  - conjunctiva 40% of cases
- Mainly Gram-positive species
  - coagulase-negative staphylococci
  - *Corynebacterium* spp.
  - others: *Propionibacterium* spp., *S.aureus, Micrococcus* spp., *Bacillus* spp.
- Gram-negative organisms isolated infrequently (<5%)

98640-37S.PPT

6L498640-37

Description of the External Ocular Biota

Organisms are recovered in low numbers from the ocular surface. Typically, the conjunctiva yields a median number of bacteria of five colony forming units (cfu) per swab.

The lids show colonization in 60% of cases and the conjunctiva in 40% of cases.

The most common species isolated are mainly Gram-positive. The isolates include:

- Coagulase-negative staphylococci
- *Corynebacterium* spp.

Less commonly isolated are:

- *Propionibacterium* spp.
- *Staphylococcus aureus*
- *Micrococcus* spp.
- *Bacillus* spp.

57

EXTERNAL OCULAR BIOTA

- Physiological variations:
  - transient/permanent biota
  - geographical regional variations
  - changes with sleep:
    - increase in Gram-positive organisms
    - no change in Gram-negative organisms

98640-38S.PPT

6L498640-38

External Ocular Biota

There is some debate as to whether the normal ocular surface has resident microbiota or whether organisms are a reflection of skin colonization.

Geographically, regional variations exist. Tropical environments have higher rates of fungal recovery. The ocular biota is also modulated by antibiotic use.

There is an increase in the number of Gram-positive organisms with eye closure.

58

FUNCTION OF MUCOSAL MICROBIOTA

- Mediate mucosal defences
- Prevent colonization by pathogens by:
  - biocompetition
  - production of antimicrobial agents
  - production of toxins

98640-39S.PPT

6L498640-39

Function of Mucosal Microbiota

In non-ocular mucosa, resident biota mediate the normal defence mechanisms by competing for nutrients or receptor sites and by producing specific antimicrobial agents or toxins specific to pathogens.

59

FUNCTION OF OCULAR MICROBIOTA

- *S.epidermidis* and *Corynebacterium* sp. may act synergistically to inhibit *S.aureus* in the nasal mucosa
- *Propionibacterium* sp. may enhance specific and local immune defence

98640-40S.PPT

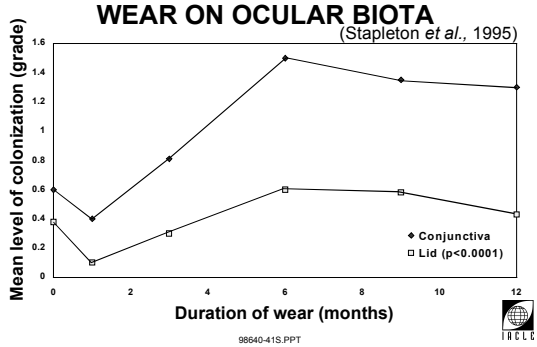
6L498640-40

Function of Ocular Microbiota

Some bacteria may be significant in preventing colonization by pathogens and in maintaining non-pathogenic biota at manageably low levels. They may also play a role in immune defence.

60

EFFECT OF DAILY CONTACT LENS WEAR ON OCULAR BIOTA



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Effect of Daily Contact Lens Wear on Ocular Biota

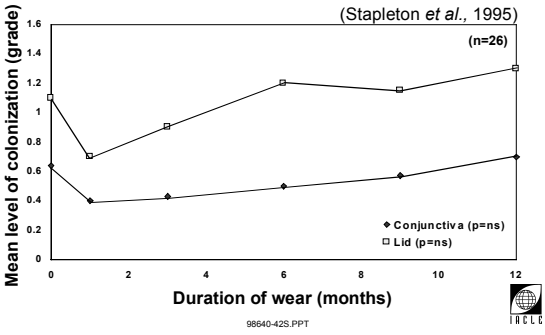
Reports suggest that there is an increase in the number of bacteria isolated from the conjunctiva and lid during daily wear of contact lenses. However, the spectrum of biota remains unchanged.

Stapleton *et al.* (1995) reported that conjunctival biota could be related to the contaminants of the contact lens storage case.

Daily wear of contact lenses may interfere with the clearing of normal ocular biota and permit an increased colonization by normal biota.

61

NEOPHYTE EW LENS USERS



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Effect of Overnight Contact Lens Wear on Ocular Biota

With overnight wear of contact lenses there is no change in the number of organisms isolated over time. However, there appears to be a shift in the spectrum of organisms. The organisms most likely to be recovered return either sterile cultures or Gram-negative staining. Overnight wear also appears to cause some alteration in the normal ocular biota over time.

Other studies have reported no differences between contact lens wear and non-wear, although increased positive cultures were shown in one study in former wearers, and also in association with certain modes of lens wear and care systems (Fleiszig *et al.* 1992).

62

EFFECT OF CONTACT LENS WEAR ON OCULAR BIOTA

Identification of Staphylococci:

- In EW most numerous isolate = S.epidermidis
- in DW most numerous isolate = S.capitis/S.warneri
- In DW/EW greatest isolation frequency = S.capitis/S.warneri
- S.haemolyticus, S.lugdenesis, S.hyicus, S.schleferi, S.intermedius, S. aureus isolated infrequently

98640-43S.PPT

6L498640-43

Effect of Contact Lens Wear on Ocular Biota

The identification of staphylococci from the eye during contact lens wear indicates that coagulase-negative staphylococci (CNS) are the commonest group of Gram-positive organisms colonizing the ocular surface.

Historically, *Staphylococcus epidermidis* was considered the most frequent CNS isolate. Studies show that other species are also important and highlight differences in CNS microbiota between daily and extended wear modalities (Leitch *et al.,* 1998).

63

CONTACT LENS BIOTA: ASYMPTOMATIC WEAR

- Lens contamination is infrequent
- Commonest organisms are CNS
- Occasional isolation of *S.aureus*, *Streptococcus* spp., *Propionibacterium* spp.
- Gram-negative bacteria rarely isolated
- No difference in frequency of isolation for DW & EW

98640-44S.PPT

6L498640-44

Contact Lens Biota: Asymptomatic Wearers

Only small numbers (usually <10 cfu per lens) of organisms are isolated from asymptomatic contact lens wearers. These organisms come from the hands, imperfect lens storage systems and the environment.

More colonization occurs on the lens than on the conjunctiva, but both sites have less than that found on the lid.

Normal contact lens biota cannot explain an increased frequency of infection and inflammation with extended contact lens wear.

During uncomplicated lens wear, organisms are cleared from the lens surface. It is a very different situation during lens-related inflammation and infection (see below).

64

CONTACT LENS BIOTA: ASYMPTOMATIC WEAR

- Contamination occurs due to CL handling
- Higher rates of contamination follow CL storage
- Main sources:
  - skin, air, pets
- Gram-negatives from water/low levels of environmental contamination

98640-45S.PPT

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65

LENS CARE PRODUCT CONTAMINATION: ASYMPTOMATIC WEAR

- Case contamination in >80% of wearers
- CNS, environmental and enteric Gram-negatives commonly associated
- *Ps. aeruginosa* rare in asymptomatic wear
- *Acanthamoeba* sp. in 7%
- Fungi in 3-5%
- Lack of association between compliance and lens care product contamination

98640-46S.PPT

6L498640-46

Lens Care Product Contamination in Asymptomatic Lens Wear

Lens case contamination has been found to be highly prevalent amongst asymptomatic wearers. Environmental, skin and enteric organisms have been recovered.

Several studies have demonstrated a lack of association between the level of compliance and lens care product contamination. This indicates that good compliance does not necessarily result in contamination-free storage cases.

66

CONTACT LENS BIOTA: CONTACT LENS INDUCED ACUTE RED EYE

- High numbers of Gram-negative bacteria recovered from the CL only
  - *H.influenza, Ps.aeruginosa, Serr.marcescens, Stenotrophomonas* spp.
- No corneal colonization by organisms
- Role of endotoxin in pathogenesis

98640-47S.PPT

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Contact Lens Biota: CLARE

In the inflammatory condition Contact Lens-induced Acute Red Eye (CLARE), which occurs in some SCL extended wearers, very high numbers of Gram-negative bacteria have been isolated from the lens.

67

CONTACT LENS BIOTA: STERILE INFILTRATES / CLPU

- CLPU associated with ocular carriage of Gram-positive bacteria especially *S.aureus*
- Toxin related?
- Sterile infiltrates associated with bacterial contamination of the storage case

98640-48S.PPT

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Contact Lens Biota: Sterile Infiltrates / CLPU

During contact lens wear, inflammatory conditions of the cornea such as sterile infiltrates and Contact Lens-induced Peripheral Ulcer (CLPU) are associated with Gram-positive bacteria.

These events are possibly associated with marginal infiltrates that can occur in non-lens wear.

68

CONTACT LENS BIOTA: CORNEAL INFECTION

- Causes of CL-associated infection
  - *Ps. aeruginosa* predominates (60-70% of culture-proven cases)
  - others; *Serratia* spp., *Proteus* spp., *Moraxella* spp., other *Pseudomonads*
  - *Acanthamoeba* spp. strongly associated with lens related infections

98640-49S.PPT

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Contact Lens Biota: Corneal Infection - LCPs

The organisms associated with lens related infections are different from those associated with non-lens related infections.

Similar or identical organisms to the causative agent have been isolated from the lens or lens storage case in contact lens-associated keratitis.

Bacterial biofilm formation is a common phenomenon in natural microbial ecosystems.

69

CONTACT LENS BIOTA: CORNEAL INFECTION - LCPs

- Causative organism isolated from care system or CL
- Lens & storage contamination can occur despite good compliance

98640-50S.PPT

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70

CONTACT LENS BIOTA: ROLE OF BIOFILM

Roles of bacterial biofilm:

- Explains unexpected persistence of organisms in storage cases
- May play a part in pathogenesis of infection

98640-81S.PPT

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Contact Lens Biota: Corneal Infection - Biofilm

Cells in biofilms are more resistant than planktonic organisms (non-adherent cells in suspension). This was initially attributed to physical exclusion. However, recent evidence shows biofilms to be heterogeneous structures comprised of stacks of slow growing bacteria interspersed with water channels. This suggests that penetration of antimicrobial compounds can occur.

Phenotypic alteration of bacteria is a more likely cause of resistance. This alteration involves modified hydrophobicity, outer membrane proteins, cell wall structure and alginate production in adherent cells.

71

CONTACT LENS BIOTA: CORNEAL INFECTION - BIOFILM

- Biofilm defined as:
  - a functional consortia of micro-organisms, organized at interfaces, within exopolymer matrix
- Organisms in biofilms resist antimicrobials by:
  - physical exclusion
  - phenotypic alterations

98640-51S.PPT

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Biofilm formation is common in wearers with bacterial and amoebic infections. This may be one explanation for the infections that occur among disposable EW SCL patients.

The greater density and increased occurrence of biofilms in contact lens cases suggests that lens storage cases may be the initial source of microbial contamination.

72

CONTACT LENS BIOTA: CORNEAL INFECTION - LENS CASES

(McLaughlin-Borlace *et al.,* 1998)

- Biofilm formation on CLs & storage cases in bacterial & amoebic infections:
  - 17/20 storage cases vs 11/20 CLs
  - greater density of biofilm in storage cases

98640-52S.PPT

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73

SUMMARY: MICROBIOLOGY AND CONTACT LENS WEAR

- Gram-positive organisms are present in low numbers on the normal ocular surface
- Some evidence exists that the ocular biota is altered by hydrogel DW/EW, but the implications of this are unclear
- In asymptomatic wearers, lens contamination is infrequent
- Instances of contamination usually reflect the normal biota

98640-79S.PPT

6L498640-79

Summary

74

SUMMARY: MICROBIOLOGY AND CONTACT LENS WEAR

- Lens-related inflammation may be due to lens colonization by Gram-negative species (CLARE) or ocular carriage of Gram-positive species (CLPU)
- CL-related microbial keratitis is predominantly bacterial, with *Ps. aeruginosa* the commonest corneal isolate

98640-80S.PPT

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Unit 6.5

(2 Hours)

Lecture 6.5: Ocular Host Defence Systems and Contact Lens Wear

Course Overview

Lecture 6.5: Ocular Host Defence Systems and Contact Lens Wear

V. Non-Specific Host Defences

VI. Specific Host Defences

VII. Contact Lens Wear and Host Defence Systems

Lecture 6.5

(2 Hours)

Ocular Host Defence Systems and Contact Lens Wear

Table of Contents

I Non-Specific Host Defences

1

OCULAR HOST DEFENCE SYSTEMS AND CONTACT LENS WEAR

98650-1S.PPT

6L598650-1

This lecture describes the major mechanisms by which the anterior eye is defended from debris and invading micro-organisms. Particular attention is paid to the role of the tear film.

The potential effects of contact lens wear on the defence systems are also examined.

2

NON-SPECIFIC HOST DEFENCES

- Lids and lashes
- Blink mechanism
- Tear flow
- Epithelial shedding/integrity
- Tear proteins

98650-2S.PPT

6L598650-2

Ocular Non-Specific Host Defence Systems

- Lids and lashes – prevent micro-organisms and debris from entering the eye.
- Blinking and tear flow – wash out micro-organisms and debris.
- Epithelial shedding – remove dead cells and micro-organisms attached to them, and debris. Epithelial integrity is an important defence mechanism. Animal studies have shown that few micro-organisms can invade and infect a cornea with an intact epithelium.
- Major and minor tear proteins.

3

MAJOR TEAR PROTEINS

- Lysozyme
- Lactoferrin
- β lysin
- Lipocalin
- Mucin

98650-3S.PPT

6L598650-3

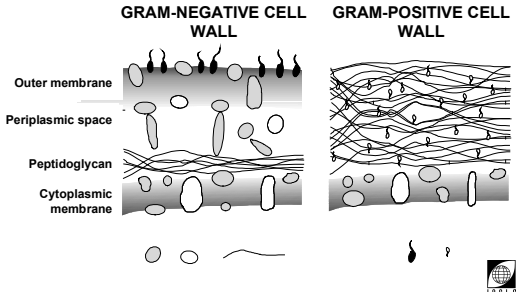
Major Proteins in the Ocular Tear Film

The major tear proteins are those that are present in milligram per millilitre (mg/mL) quantities.

They are all involved in protecting the eye against foreign bodies, including micro-organisms.

4

BACTERIA: CELL WALLS



98640-25S.PPT

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Bacterial Cell Membranes

Bacterial cell membranes (like all cell membranes) are composed of a bilayer of lipids (phospholipids). These are stable under isotonic conditions. However they easily rupture if the salt concentration on the outside of the bilayer is less than that inside, a situation that is nearly always the case with living organisms.

Therefore, bacteria need to be able to prevent death by lysis of their membranes. They achieve this by synthesizing a substance called peptidoglycan on the outside of their lipid bilayer. This provides a rigid layer that prevents lysis. The amount of peptidoglycan that bacteria contain varies, and this variation is the basis for differentiating bacteria into

Gram-positives (more peptidoglycan) and Gram-negatives (less peptidoglycan and an additional lipid bilayer).

Refer to Lecture 6.4: Microbiology for a more detailed discussion of bacteria**.**

5

LYSOZYME

- Is an enzyme, muramidase
- Catalyes the hydrolysis of N-acetyl muramic acid and N-acetyl glucosamine
- More active against Gram-positive bacteria than Gram-negatives

98650-5S.PPT

6L598650-5

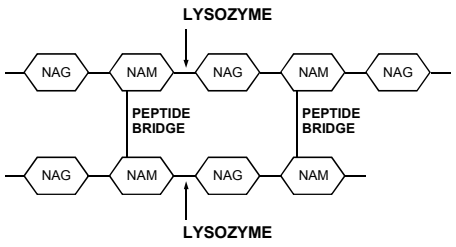
Action of Lysozyme on Bacteria

Lysozyme is an enzyme, muramidase, that catalyses the hydrolysis of the linkage between N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG), two sugars that make-up the cell wall (peptidoglycan) of susceptible bacteria (slide 6).

Lysozyme is more active against Gram-positive bacteria. Gram-negative bacteria possess an outer lipid bilayer/membrane that prevents lysozyme from reacting with their peptidoglycan.

6

LYSOZYME ACTION ON BACTERIA



98650-4S.PPT

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7

LACTOFERRIN

- Chelates Fe^{3+} ions:
  - essential for bacterial growth
- Disrupts outer membrane of gram-negatives:
  - chelates Mg^{2+}/Ca^{2+} or binds to lipopolysaccharide

98650-6S.PPT

6L598650-6

Action of Lactoferrin on Bacteria

Bacteria need nourishment to survive. Many have extremely limited nutritional requirements such as a simple source of carbon (e.g. glucose) and nitrogen (e.g. ammonia). Other bacteria require more complex foods such as sugars other than glucose, and amino acids as nitrogen sources. In addition to these food sources, bacteria, like humans, require vitamins and minerals.

Lactoferrin chelates (literally means claws but 'binds' is the actual result) ferric (Fe^{3+}) ions that are an essential element for bacterial growth (required for a variety of enzyme functions).

It may also disrupt the outer membrane of Gram-negative bacteria by chelating magnesium (Mg^{2+}) ions and calcium (Ca^{2+}) ions or by binding directly to the outer membranes by an electrostatic interaction, i.e. by the attraction between opposite charges.

Lipopolysaccharide (LPS) in the outer membrane is negatively charged (–ve) and lactoferrin has a positively charged (+ve) region called lactoferricin.

8

β LYSIN AND LIPOCALIN

- Reported to be antibacterial
- Action unknown

98650-7S.PPT

6L598650-7

Action of βLysin and Lipocalin on Bacteria

At present the mechanisms by which these substances act on bacteria are unknown. Further investigation is required to elucidate their role in maintaining the health of the anterior eye.

9

MUCIN

- Bacteria bind via lectin/carbohydrate interactions
- Prevents bacterial adhesion by acting as a non-specific blocker
- Aids in removal by tears and/or blink mechanism

98650-8S.PPT

6L598650-8

Action of Mucin on Bacteria

Bacteria bind strongly to mucin, usually via a lectin (protein) on the surface of the bacteria and a carbohydrate residue (such as galactose) on the mucin.

Mucin may help to remove bacteria by:

- Preventing bacterial adhesion by acting as a non-specific blocker of adhesion to epithelial surfaces.
- Facilitating removal by tears and/or blinking.

10

EFFECT OF SLEEP ON NON-SPECIFIC COMPONENTS OF TEARS

Sack *et al.*, 1992

Protein	Tear Type	Concentration (mg/mL)	% Total tear protein (mean ± SD)
Total	Reflex	6 ± 0.8	
	Closed Eye	18 ± 6.2	
Lactoferrin	Reflex	1.8	30 ± 10
	Closed Eye	1.8	10 ± 10
Lysozyme	Reflex	1.6	26 ± 4
	Closed Eye	1.8	10 ± 4

98650-9S.PPT

6L598650-9

Sleep and Non-Specific Tear Proteins

The lactoferrin and lysozyme proteins in the tears are regulated and their concentration does not vary with changes in tear secretion rates.

Sack *et al.* (1992) demonstrated that the concentrations of lactoferrin and lysozyme do not change during sleep (i.e. they remain between 1.6 and 1.8 mg/mL). However, the relative concentrations of these proteins, i.e. the percentage of the total tear protein they represent, decreases (30 to 10 and 26 to 10 respectively) as the total protein increases dramatically (6 to 18) with sleep-induced closed eye (see data in slide 10).

11

MINOR TEAR PROTEINS INVOLVED IN HOST DEFENCE

- Enzymes and enzyme inhibitors
- Complement
- Cytokines/chemokines
- Arachidonic acid metabolites

98650-10S.PPT

6L598650-10

Minor Tear Proteins and Host Defence Systems

These tear proteins (usually in micrograms per millilitre [μg/mL] or less concentrations) generally increase in concentration during sleep. They have various regulatory functions including:

- Destroying micro-organisms.
- Coating entrapped debris and micro-organisms (opsonization) to make them more susceptible to engulfment by phagocytic cells (complement proteins coat debris and micro-organisms).
- Recruitment of white blood cells to the tear film (chemokines, complement, arachidonic acid metabolites).

- Activation of white blood cells (cytokines, complement, arachidonic acid metabolites).
- Vasodilation (complement, cytokines, arachidonic acid metabolites).

The complement proteins are involved in a major effector pathway for the inflammation response in the anterior eye. Two pathways of activation are described:

- The Alternative Pathway.
- The Classical Pathway.

12

MINOR TEAR PROTEINS: ANTIMICROBIAL ENZYMES and ENZYME INHIBITORS

- Secretory Phospholipase A_2
- Specific Leukocyte Protease Inhibitor (SLPI)
- Elafin

98650-75S.PPT

6L598650-75

Minor Tear Proteins

Recently, secretory phospholipase A_2 has been shown to be the principal staphylococcal bactericide in tears (Qu and Lehrer, 1998). Secretory phospholipase A_2 acts by digesting the phospholipids in the bilayer membranes of bacteria.

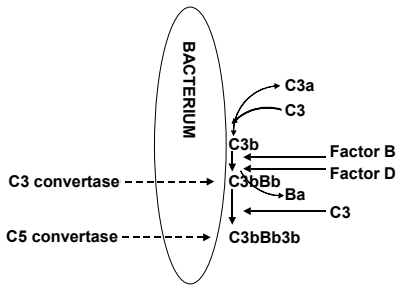
Specific Leucocyte Pretease Inhibitor (SLPI) and Elafin are both potent anti-proteases, i.e. they inhibit the action of proteases that might degrade the cornea, especially the stromal collagen. Their antimicrobial action is not well understood but may be related to their anti-protease activity.

Proteases are important enzymes that liberate amino acids from proteins. Microbes then use these as nutrients. The inhibition of microbial proteases prevents microbial growth.

SLPI is also a cationic protein that may disrupt the outer membrane of Gram-negative bacteria.

13

ALTERNATIVE PATHWAY



98650-11S.PPT

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The Alternative Pathway Mechanism

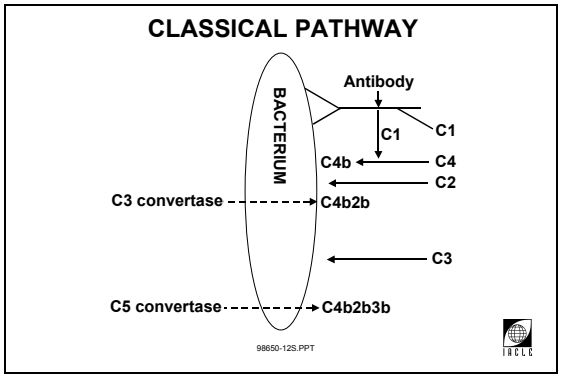
The major activators of the Alternative Pathway are micro-organisms or debris. In this pathway, Complement (C3) binds to hydroxyl (OH) or amide (NH) groups on carbohydrates or proteins.

Upon binding, C3 is converted to C3a, which is released (and which can contribute to vasodilation) and C3b, which remains attached to the surface.

After C3b attachment, factors B and D interact to form C3bBb which is the C3 convertase enzyme. This enzyme rapidly converts more C3 to C3a and C3b.

The interaction of C3bBb and further C3b molecules forms the C5 convertase enzyme which is the first step in the Terminal pathway of complement activation (see slide 15).

14



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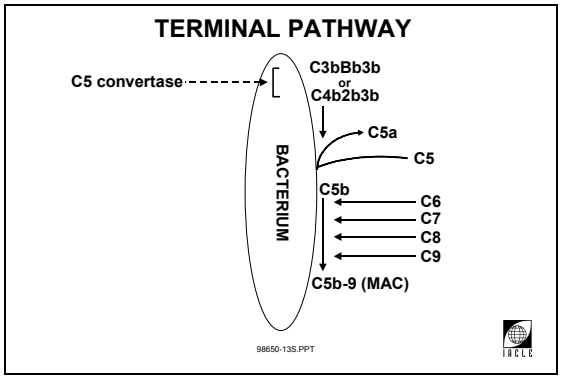
The Classical Pathway Mechanism

The immunoglobulins G and M (IgG and IgM) are the major activators of the Classical Pathway, the pathway that is dependent on antibody/antigen interactions. It is more rapid than the Alternative Pathway mechanism.

The C3 convertase (C4b2b) in this pathway is formed by the interaction of C1 bound to an antibody, and C4 and C2. Again, interaction of this enzyme with further C3 molecules forms the C5 convertase, the first step in the Terminal Pathway (see slide 15).

The Classical Pathway may not function in tears, as only low levels of the key components (C1) and IgG/IgM, are found in them.

15



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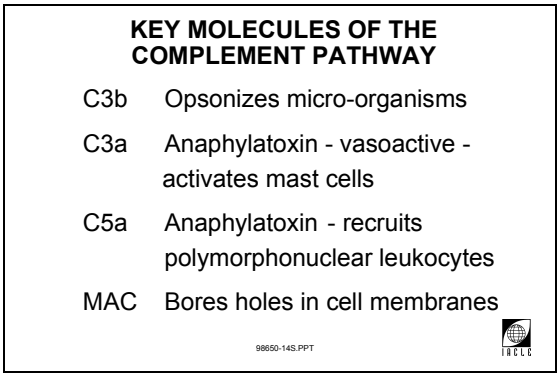
The Terminal Pathway Mechanism

Both the Alternative and Classical pathways converge at the C5 level, i.e. the formation of the enzyme c5 convertase. The C5 convertases activate C5 and precipitate the recruitment of C6, 7, 8 and multiple C9 molecules to form the Membrane Attack Complex (MAC).

To destroy invaders, the MAC bores holes into biological membranes, including those of micro-organisms. Once a membrane is perforated the contents of the cell can leak, cell viability is compromised and cell death ensues.

16



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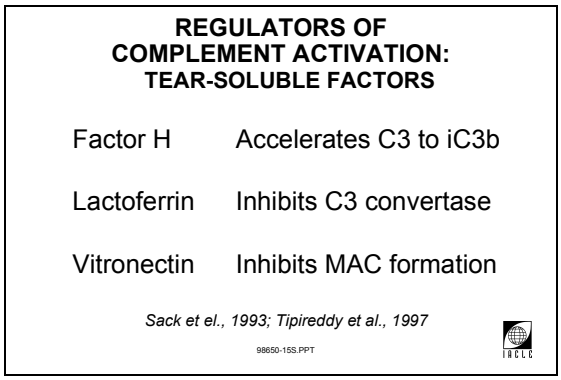
Components of the Complement Pathway

The activation of complement opsonizes (coats) micro-organisms or debris for phagocytosis by white blood cells (PolyMorphoNuclear leukocytes (PMNs) or macrophages). It releases the anaphylatoxins that:

- Recruit PMNs to the site of activation.
- Cause vasodilation.
- Activate mast cells to release inflammatory mediators such as histamine.

Complement activation also produces the membrane attack complex (MAC) that can destroy invading micro-organisms.

17



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Regulators of Complement Activation: Tear Soluble Factors

Regulation of complement is critical to prevent damage to mammalian cell membranes. A number of regulators act on the Complement Pathway. These include:

- Factor H, which accelerates the cleavage of C3 to iC3b (an inactive form of C3b that can not form the C3 convertase enzyme), preventing further complement activation in the Alternative Pathway.
- Lactoferrin, which inhibits classical C3 convertase.
- Vitronectin, which inhibits the formation of membrane attack complexes (MACs).

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REGULATORS OF COMPLEMENT ACTIVATION: CELL-BOUND FACTORS

DAF	Stimulates C3 convertase decay
C8bp	Prevents C8/C9 binding to MAC
CD-59	Prevents MAC formation

98650-16S.PPT

6L598650-16

Regulators of Complement Activation: Cell-Bound Factors

The cell-bound factors that regulate complement activation include:

- Decay Accelerating Factor (DAF) which accelerates C3 convertase decay.
- C8 Binding Protein (C8bp) which prevents C8 and C9 binding to MACs.
- CD-59 that prevents the formation of membrane attack complexes (MACs).

19

EFFECT OF SLEEP ON COMPLEMENT

Protein	Tear Type	Concentration µg/mL (mean ± SD)	% Plasma concentration
C3	Reflex	4.0 ± 5.6	0.6
	Closed Eye	107 ± 84	15
Factor B	Reflex	0.1 ± 0.1	0.1
	Closed Eye	21 ± 8	20
C5	Reflex	0	0
	Closed Eye	1.0 ± 0.4	1.9

Willcox *et al*., 1997

98650-17S.PPT

6L598650-17

Effect of Sleep (Closed Eye) on the Complement System

Both Complement C3 and Factor B are prominent in closed-eye tears and indicate that the Alternative Complement Pathway probably predominates.

The increased levels of C3 and Factor B after sleep (Willcox *et al.,* 1997) indicate these may be synthesized locally, whereas C5, at only 2% of its plasma concentration, probably enters the tears by leakage of serum through the conjunctival blood vessel walls.

Other components are also at approximately 2% of their plasma level. Therefore, they all probably enter by leakage of plasma.

20

ROLE OF COMPLEMENT IN TEARS AND THE ANTERIOR EYE

- In tears:
  - recruit PMNs
  - opsonizes entrapped bacteria
- In the tissues:
  - tissue rejection after corneal grafting

98650-18S.PPT

6L598650-18

Role of Complement

The main roles of complement in tears are probably to:

- Coat micro-organisms.
- Allow phagocytosis by white blood cells.
- Activate the Classical Pathway by assisting the action of antigen/antibody complexes during tissue rejection.

21

CYTOKINES FOUND IN TEARS

- Interleukins: IL - 1 and IL - 6
- Colony stimulating factors: GM-CSF
- Growth factors: TGFβ and HGF
- Interferons: IFNγ

98650-19S.PPT

6L598650-19

Cytokines Found in the Tear Film

Many types of cell can produce cytokines. These are small proteins that affect cell growth and activation. Examples of this group include:

- The interleukins. Interlukins are small proteins that were originally thought to only signal between white blood cells (leukins).
- Colony Stimulating Factors (CSFs), e.g. Granulocyte-Monocyte Colony Stimulating Factor (GM-CSF).
- Growth factors, e.g. Tissue Growth Factor beta (TGFβ) or Hepatocyte Growth Factor (HGF).
- Interferons, e.g. InterFeroN gamma (IFNγ).

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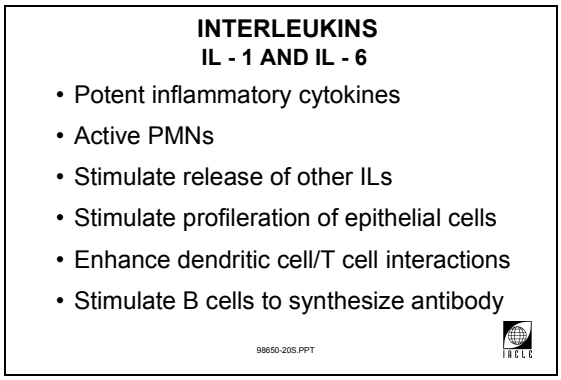

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Interleukins and Inflammation

The interleukins play a number of roles in the inflammatory response. These include:

- Preparation of the PMN for phagocytosis.
- Stimulation of release of each other.
- Involvement in the wound healing response (especially IL-6).
- The Dendritic cells (see slide 54) 'present' antigen to T cells (see slide 53) and stimulate immune responses, especially B cell (immunoglobulin) and cytotoxic T cell responses. Cytotoxic T cells are specialized white blood cells that can kill mammalian cells infected with micro-organisms. These cells are defective in disease such as human immunodeficiency virus (HIV-AIDS).
- Involvement in the production of specific immunoglobulins in the eye, especially IgA.

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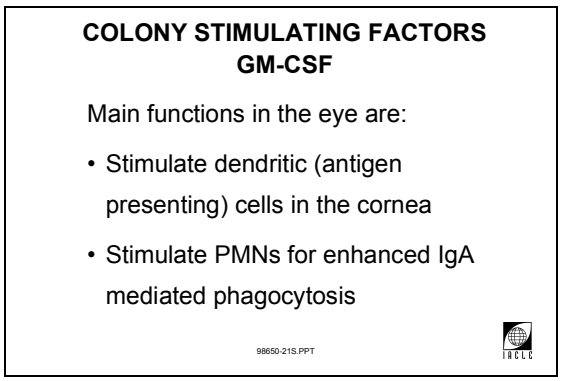

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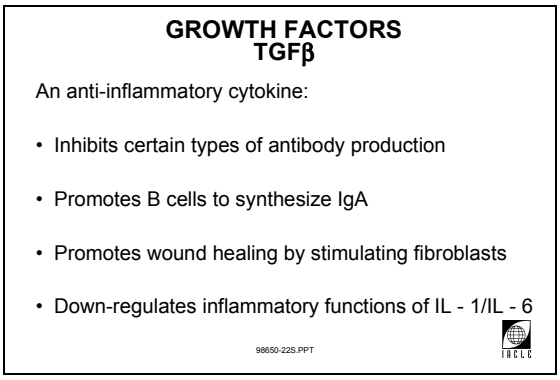
Colony Stimulating Factors and Inflammation

Only one colony-stimulating factor, Granulocyte-Monocyte Colony Stimulating Factor or GM-CSF, has been reported in tears. In the eye, GM-CSF acts on resident antigen-presenting cells (dendritic cells) and recruited PMNs.

24



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Growth Factors and Inflammation

Tissue Growth Factor β is probably a very important cytokine in the eye as it has the effect of controlling or dampening the inflammatory system that is based on other cytokines.

25

INTERFERONS
IFNγ

In the eye this cytokine:

- Influences antigen presentation to T cells
- Activates phagocytes
- Inhibits IgE production

98650-23S.PPT

6L598650-23

Interferons and Inflammation

Unlike other interferons, IFNγ is not anti-viral. It is a pro-inflammatory cytokine.

26

CHEMOKINES FOUND IN TEARS

IL - 8

- A specific attractant for PMNs
- Produced by epithelial cells

98650-24S.PPT

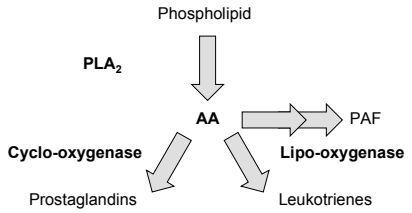
6L598650-24

Chemokines Found in the Tear Film

IL-8 has been found in the tear film. This chemo-attractive cytokine (chemokine) is believed to be the major effector for PMN recruitment into the tears during sleep and into the stroma during infection/inflammation.

27

ARACHIDONIC ACID METABOLITES



98650-25S.PPT

6L598650-25

Arachidonic Acid Metabolites

The membrane-bound phospholipid arachidonic acid is in all cells in the body. Metabolites can be released by PhosphoLipase A_2 (PLA_2) by a diverse array of signals including bacterial adhesion and cytokines.

There are two major pathways for activation of arachidonic acid.

- Cyclo-oxygenase Pathway: produces prostaglandins, thromboxanes and prostacyclins that increase vascular dilation and permeability.
- Lipo-oxygenase Pathway: produces leukotrienes that are chemo-attractants for PMNs and macrophages.

The platelet activation factor (PAF) plays a role in the activation of PMNs and increases vascular permeability.

28

POSSIBLE FUNCTIONS OF ARACHIDONIC ACID METABOLITES IN THE TEARS

• Prostaglandins	increased vasodilation/vascular permeability
• Leukotrienes	chemo-attractant for PMNs and macrophages, stimulates PMNs
• PAF	activation of PMNs, vascular permeability

98650-26S.PPT

6L598650-26

29

EFFECT OF SLEEP ON CYTOKINES AND ARACHIDONIC ACID METABOLITES

Cytokines/AAs	Tear Type	Concentration (pg/mL)
IL - 6	Reflex	0
	Closed Eye	150 ± 110
IL - 8	Reflex	2000 ± 2000
	Closed Eye	$150x10^3 \pm 100x10^3$
LTB_4	Reflex	232 ± 35
	Closed Eye	1005 ± 205

98650-27S.PPT

6L598650-27

Effect of Sleep (Closed Eye) on Cytokines and Arachidonic Acid Metabolites

Thakur *et al*. (1998) found that cytokines and arachidonic acid metabolites are only present in the tear film in significant amounts (at active levels) during sleep. IL-8 is probably the major chemo-attractant, recruiting PMNs into the tear film during closed eye.

The high concentration of IL-6 and LTB_4 in closed-eye tears probably primes the PMNs for phagocytosis.

30

WHITE BLOOD CELL TYPES INVOLVED IN NON-SPECIFIC DEFENCES OF THE ANTERIOR EYE

98650-28S.PPT

6L598650-28

White Blood Cells and Ocular Defence Mechanisms

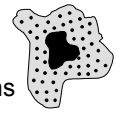
The anterior eye is unusual in that the cornea is avascular.

Resident cells, as well as recruited cells in the conjunctiva and cornea, are involved in non-specific host defence.

31

MACROPHAGES

- Resident white blood cells
- Phagocytose micro-organisms
- Kill micro-organisms
- Signal for recruitment of other cells
- Found in all tissues - sparse in cornea



98650-29S.PPT

6L598650-29

Macrophages

Macrophages are one of the primary cells involved in the ocular defence mechanism. These cells can phagocytose micro-organisms that may or may not be opsonized (coated with complement or antibody).

One of the main functions of macrophages is to ingest and kill invading micro-organisms. They are equipped with a variety of secretory products that aid in the killing process.

32

PHAGOCYTE SECRETORY PRODUCTS

- Enzymes - lysozyme
  - protease
- Complement components
- Cytokines and arachidonic acid metabolites
- Reactive oxygen radicals - H_2O_2
  - O_2^-
  - OH^-

98650-30S.PPT

6L598650-30

Phagocyte Secretory Products

Complement and cytokines act as described previously. Some of the most powerful killing agents are the oxygen radicals. These agents oxidize components of micro-organisms and prevent them from functioning.

The oxygen radicals are:

- Hydrogen peroxide (H_2O_2).
- Superoxide (O_2^-).
- Hydroxide (OH^-).

33

RECRUITED WHITE BLOOD CELLS INVOLVED IN NON-SPECIFIC HOST DEFENCE

98650-31S.PPT

6L598650-31

34

THE POLYMORPHONUCLEAR LEUKOCYTE

- Phagocytose micro-organisms
- Kill micro-organisms
- Signal for further PMN recruitment

98650-32S.PPT

6L598650-32

The Polymorphonuclear Leukocyte

The polymorphonuclear leukocyte (PMN) or neutrophil is the main effector cell in the eye.

The PMN is a highly granulated leucocyte (i.e. small particles are seen within the cell after appropriate staining). It is usually the first cell type recruited by macrophages, or other cells, to sites of tissue injury or infection.

Chemotactic factors involved in this recruitment are IL-8, LTB_4, complement C5a and microbial peptides.

PMNs can release chemotactic substances to recruit further cells.

35

PMN SECRETORY PRODUCTS
MICROBICIDAL PROTEINS

Azurophillic granules	Specific granules
Defensins	Lysozyme
Myeloperoxidase	NADPH oxidase
Lysozyme	Lactoferrin

98650-33S.PPT

6L598650-33

PMN Secretory Products

Defensins are highly positively charged small proteins that attack microbial membranes.

Myeloperoxidase and Nicotinamide Adenine Dinucleotide PHosphate (NADPH) oxidase are involved in the production of oxygen radicals.

Lysozyme and lactoferrin function as described previously.

PMNs also contain oxygen radicals.

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PMN RECRUITMENT DURING SLEEP

Tan *et al*., 1993

Tear Type	Number of PMNs
Reflex / open	0
3 h sleep	41 ± 63
8 h sleep	6583 ± 2354

98650-34S.PPT

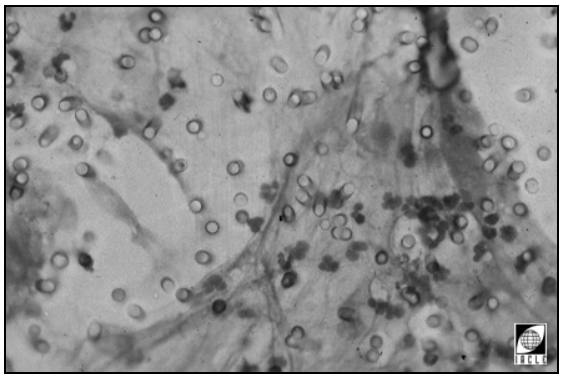
6L598650-34

PMN Recruitment During Sleep (Closed Eye)

PMNs are the principal white blood cells that enter the tear film during sleep and 'patrol' the ocular surfaces to protect against invading micro-organisms.

The number of PMNs begins to increase after three hours of sleep and thousands of PMNs can be collected from the eye after eight hours (Tan *et al*., 1993). They are cleared rapidly from the tear film after waking and the commencement of the blink reflex.

37



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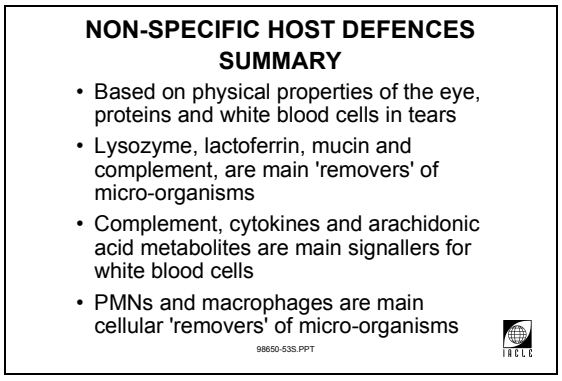
Slide 37 shows PMNs from the tear film trapped on a membrane after filtering an eye-wash solution. The PMNs are associated with mucous strands, a common finding upon eye opening following a period of sleep.

38



6L598650-53

II Specific Host Defences

39

SPECIFIC HOST DEFENCES

- Based on antibody production and T cells
- Humoral immune response based on antibodies (immunoglobulins)
- Cell mediated response based on T cells

98650-35S.PPT

6L598650-35

Specific Host Defence Mechanisms

Specific host defences are based around the production of antibodies. These are proteins synthesized to react against specific antigens.

Also involved in specific host defences are lymphocytes, one of the types of white blood cells.

Two types of lymphocytes are of interest. The T cells have a role in detecting foreign antigens, while the B cells secrete immunoglobulins.

Many functions of the specific host defence system react in concert with the non-specific host defence systems.

40

FUNCTION OF ANTIBODIES

- Bind to surface of micro-organisms to prevent adhesion to host surfaces
- Neutralize toxins
- Aid in phagocytosis
- Activate complement
- Activate specific white cells

98650-36S.PPT

6L598650-36

Function of Antibodies

Antibodies respond rapidly to micro-organisms that threaten the eye. However, if specific antibodies are to be produced, the eye must have been exposed to the micro-organism previously.

41

THE IMMUNOGLOBULINS FOUND IN TEARS

- IgA
- IgG
- IgM
- IgE

98650-37S.PPT

6L598650-37

Immunoglobulins in the Tear Film

Immunoglobulins provide the host with exquisite sensitivity and specificity in detecting and combating the many different antigens that the eye may encounter.

42

IMMUNOGLOBULIN A

- Most abundant immunoglobulin in tears
- Two isoforms, IgA and IgA_2
- Both forms at equal concentrations
- Both forms have similar functions
- IgA_2 is resistant to bacterial proteases

98650-38S.PPT

6L598650-38

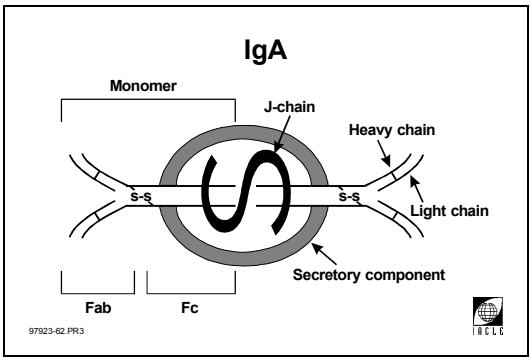
Immunoglobulin A (IgA)

IgA is composed of two heavy and two light chains. The whole molecule is a dimer (two full immunoglobulin molecules; a heavy and a light chain) joined by a J-chain (slide 43). During secretion, the secretory component is added.

One portion, the Fab portion, is specific to different antigens whilst the other, the Fc portion, is recognized by white blood cells.

The Fab portion is the smallest after digestion with enzymes that hydrolyze amine bonds and contains an intact light chain but a degraded heavy chain.

43



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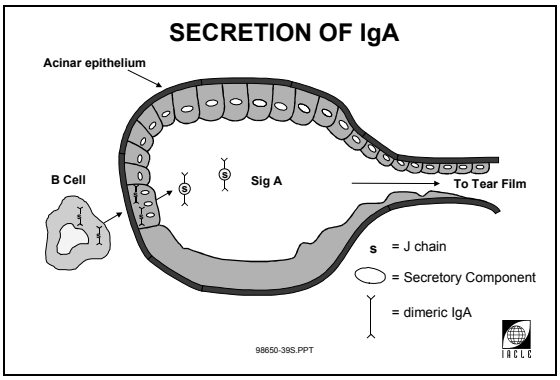
The Fc portion is the largest piece after digestion with enzymes that hydrolyze amine bonds and contains only part of the heavy chain.

44



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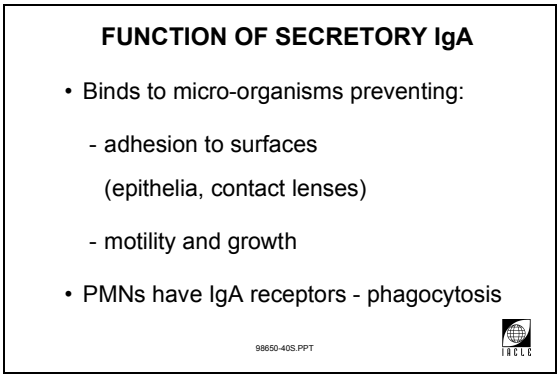
Secretion and Function of Immunoglobulin A

B cells, which are specialized immunoglobulin secreting cells, are present in the lacrimal ducts. These cells secrete IgA linked to the J-chain.

Before secretion into the tear film, IgA associates with a secretory component on the basolateral surface of acinar cells (slide 44).

45



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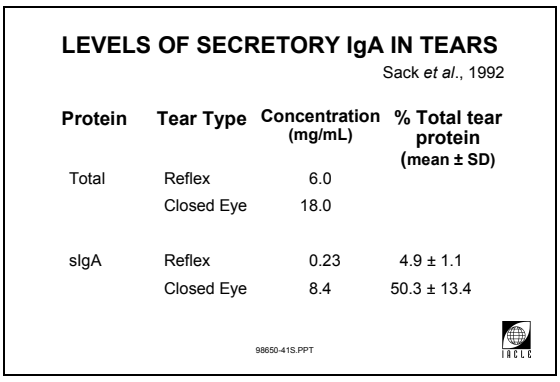

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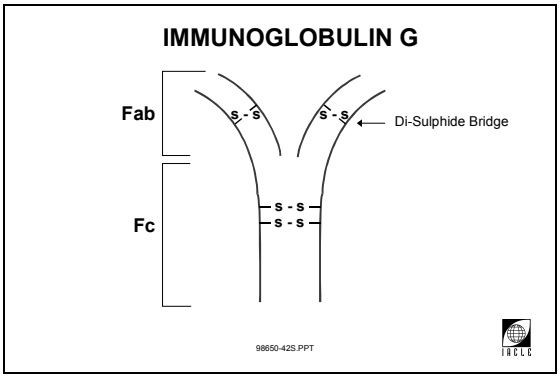
Level of sIgA in the Tear Film

The protein sIgA is a constitutively secreted protein. Its concentration alters with changing tear secretion levels.

The level of sIgA in the tear film is at its lowest level in reflex tears. It increases dramatically from open-eye to closed-eye tears and accounts for the vast majority of total protein in the closed-eye tear film.

47



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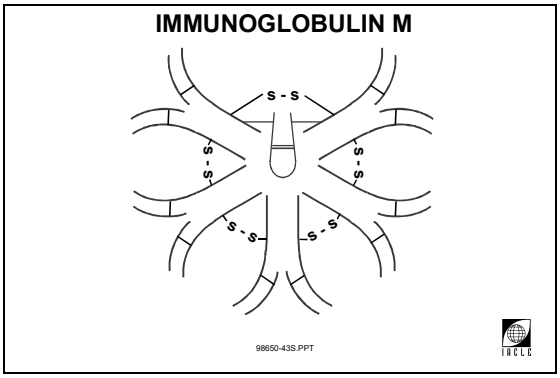
Immunoglobulin G

There are four different subclasses of Immunoglobulin G (IgG). The most abundant in blood is IgG1. It probably enters the tears by plasma 'leakage' across conjunctival blood vessels. It may also be synthesized in the lacrimal gland.

The key characteristics of IgG are:

- It activates complement via the Classical Pathway.
- It is an opsonin.
- PMNs and macrophages have IgG receptors that participate in phagocytosis.
- It is active in tissue rejections such as in corneal transplantation.

48



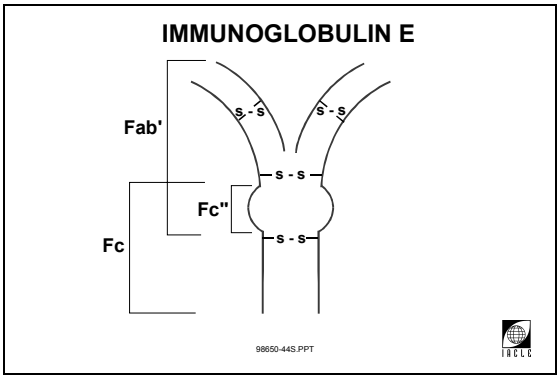
6L598650-43

Immunoglobulin M

IgM is a pentameric immunoglobulin. This means that it contains five full immunoglobulin molecules including heavy and light chains. It occurs in very low amounts in tears. It probably enters the tear film via plasma leakage.

IgM is an excellent complement activator and is active in phagocytosis.

49



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Immunoglobulin E

IgE is a monomeric immunoglobulin like IgG and its specialized function is to remove parasites.

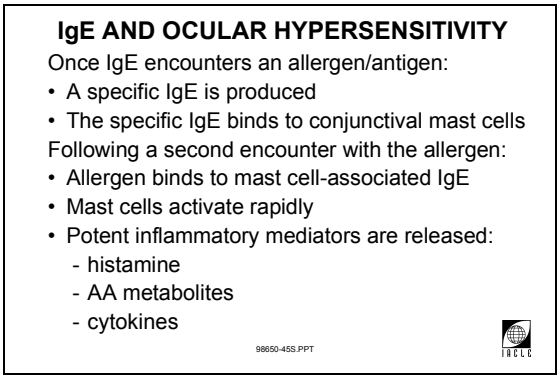
IgE is involved in many allergic disorders (hypersensitivity reactions). It interacts with specific types of white blood cells (mast cells and basophils).

Ocular hypersensitivity reactions can occur in contact lens wear as certain substances in lens cleaning fluids may be allergenic. Ocular responses during hay-fever episodes are clear examples of IgE/mast cell-mediated ocular hypersensitivity.

The responses include:

- Itching.
- Lacrimation.
- Redness.
- Chemosis.
- Lid oedema.

50



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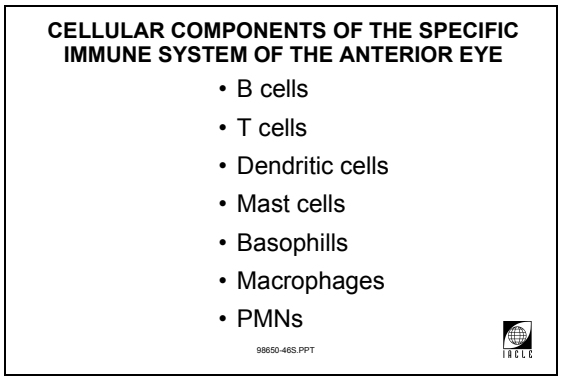

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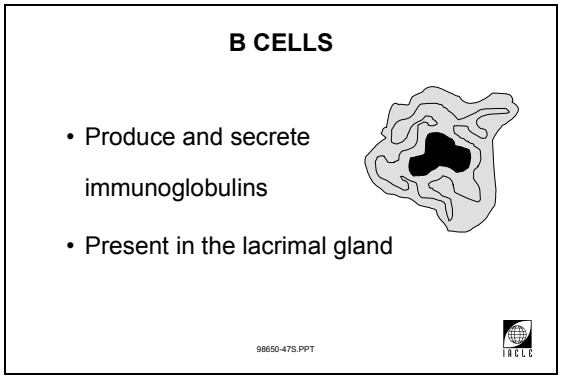
Cellular Components of the Specific Host Defence System

52



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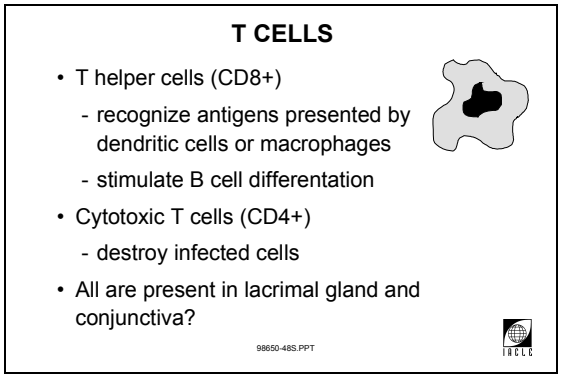
B Cells

B cells are so-named because they were initially thought to be cells derived only from bone marrow.

The principal role of the B Cells is to produce and secrete immunoglobulins. They have been identified in the lacrimal gland.

53



6L598650-48

T Cells

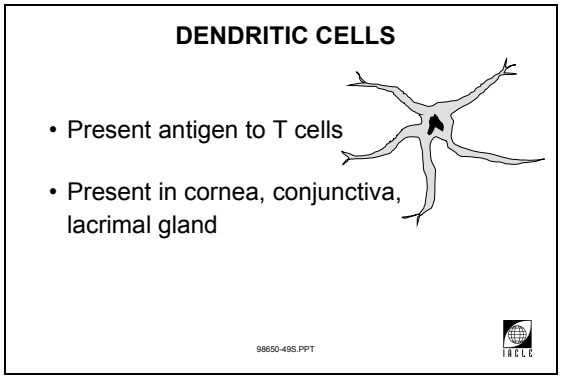
T cells are so-named because they were initially thought to be cells derived only from the thymus.

The CD (clusters of differentiation) classification of white blood cells is based on surface antigens.

There is clear evidence of the presence of all types of T-cells in the lacrimal gland, but less substantial evidence for all types in the conjunctiva.

The cornea has not been shown to have T-cells present under normal circumstances. However, T-cells may enter the cornea upon infection with viruses.

54



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Dendritic Cells

Dendritic cells have characteristic long finger-like projections radiating throughout the tissue in which they are found. Through these projections, they are in contact with neighbouring cells.

Dendritic cells are essentially tissue-associated macrophages. In normal circumstances they are present only in the periphery of the cornea. They are also found in the conjunctiva and the lacrimal gland.

Contact lens wear in animals has been shown to affect the distribution of dendritic cells in the cornea. The cells tend to move toward the centre of the cornea.

55

MACROPHAGES

- Phagocytic
- Present antigens to T cells
- Present in conjunctiva, lacrimal gland and cornea (?)

98650-50S.PPT

6L598650-50

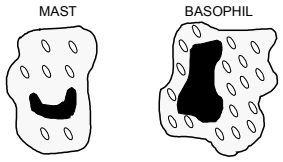
Macrophages

Macrophages are primary defensive cells and originate as bone marrow pro-monocytes. Macrophages are present throughout connective tissue and are associated with the basement membrane of small blood vessels.

56

MAST CELLS AND BASOPHILS

- Involved in allergic reactions
  - stimulated by IgE
- Present in conjunctiva - mast cells



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6L598650-51

Mast Cells and Basophils

Mast cells can be stationary in the conjunctiva, whereas basophils are recruited. Mast cells release potent inflammatory mediators, including histamine and are involved in hypersensitivity responses (IgE mediated).

57

POLYMORPHONUCLEAR LEUKOCYTES

- Recognize and destroy:
  - immunoglobulin-coated particles
  - micro-organisms

98650-52S.PPT

6L598650-52

Polymorphonuclear Leukocytes

As described previously, the PMNs are highly capable of destroying immunoglobulin-coated particles and micro-organisms.

58

SPECIFIC HOST DEFENCES SUMMARY

- Based on production of immunoglobulins
- sIgA is main antibody in tears
- IgE is main antibody in allergic reactions
- Prevent micro-organisms colonizing the eye and aid in recognition of micro-organisms by cellular components

98650-71S.PPT

6L598650-71

59

SPECIFIC HOST DEFENCES SUMMARY

- Dendritic cells signal T cells that micro-organisms are present
- T cells signal B cells to produce specific antibody
- PMNs and macrophages ingest and kill micro-organisms coated with antibody and/or complement

98650-72S.PPT

6L598650-72

III Contact Lens Wear and the Host Defence Systems

60

EFFECT OF CL WEAR ON THE ANTERIOR OCULAR HOST DEFENCES

Contact lens wear may:

- Disrupt the tear film
- Affect epithelial integrity/shedding
- Alter the balance of tear proteins
- Affect PMN recruitment during sleep
- Affect levels of immunoglobulins
- Provide a niche for bacterial colonisation and thus predispose to infection and inflammation

98650-55S.PPT

6L598650-55

Contact Lens Wear and Ocular Defence Mechanisms

Contact lenses may cause significant changes in the host defence mechanisms that protect the eye. It is important to understand all the potential effects of contact lens wear and to minimize the associated risks for each patient.

61

THE EFFECT OF CL WEAR ON EPITHELIAL INTEGRITY AND SHEDDING RATES

- Low Dk/t lenses increase levels of LDH in tears
- Low Dk/t lenses increase corneal swelling and therefore increase 'space' between cells
- Soft lenses tend to trap sloughed cells
- Low levels of mechanical abrasion may disrupt epithelium

98650-56S.PPT

6L598650-56

Epithelial Integrity and Cell Shedding Rates

Lactate dehydrogenase (LDH) is an intracellular enzyme. The presence of LDH in tears indicates that epithelial cells are damaged, as cell damage is the only mechanism by which this enzyme is released.

Corneal oedema (swelling) associated with low oxygen transmissibility (Dk/*t*) contact lenses increases the spaces between adjacent epithelial cells and can facilitate penetration of the epithelium by micro-organisms. There is some evidence that low Dk/t lenses can affect the surface of epithelial cells making them more susceptible to bacterial adhesion.

Soft lenses generally have little tear exchange and tend to trap sloughed-off epithelial cells. Bacteria adhere to these entrapped cells and are then able to penetrate the epithelium.

An advantage of RGP lenses is that the significant tear exchange that occurs with each blink flushes away the sloughed-off cells.

Low level mechanical abrasion of the epithelium by contact lenses may increase bacterial adhesion to the cells.

62

CL WEAR MAY ALTER THE BALANCE OF NON-SPECIFIC DEFENCE PROTEINS IN TEARS

- Lenses adsorb and absorb proteins from tears
- Lysozyme/lactoferrin/mucin levels do not alter
- Absorbed protein provides:
  - substratum for bacterial adhesion
  - potential for immunological reactions as protein may be 'seen' as foreign

98650-57S.PPT

6L598650-57

Tear Protein Levels

Contact lenses can adsorb and absorb proteins from the tear film. This is particularly true for soft lens materials, especially ionic high water content hydrogels. However, studies have shown that the effect on the concentration of lysozyme, lactoferrin and mucin in the tears of contact lens wearers is minimal.

The protein on, and in a contact lens has the potential to provide a substratum for the attachment of bacteria.

As the protein on a lens denatures, it can initiate a significant immunological reaction in which the material is recognized as a substance that is foreign to the body.

63

EFFECT OF CL WEAR ON CYTOKINES AND ARACHIDONIC ACID METABOLITES

Subject	IL-8	IL-6	GM-CSF	LTB_4
NCLW	150 x 10^3	150	66	1005
EWCL	230 x 10^3	218	59	1150

No statistical effect on these important inflammatory mediators

98650-58S.PPT

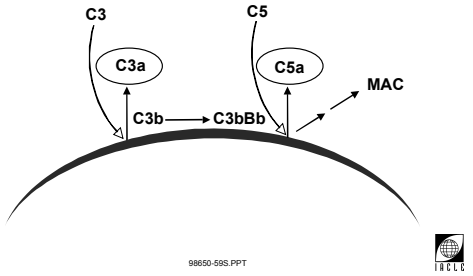
6L598650-58

Cytokines and Arachidonic Acid Metabolites

The results of an unpublished study comparing the tear concentration of cytokines and arachidonic acid metabolites in non-contact lens wearers (NCLW) and extended wear (overnight) contact lens wearers (ECLW), found that there was no significant difference between the two groups (Willcox MDP, personal communication).

64

COMPLEMENT ACTIVATION ON CLs



98650-59S.PPT

6L598650-59

Complement Activation on Contact Lenses

Contact lenses can activate the complement system. As can be seen in slide 64, C3 is activated on the surface of the lens. The activation of C3 releases C3a. This causes vasodilation and may also stimulate mast cell degranulation. C3b attached to lenses may stimulate PMNs to react with the lens surface.

Complement activation, mast cell degranulation and PMN recruitment would increase the likelihood of an inflammatory reaction.

However, C3 is at low levels and most wearers do not suffer from acute inflammation by simply wearing contact lenses. Other factors like bacterial colonization are required, and the large amounts of the complement inhibitor lactoferrin present in the tears may prevent complement activation.

Low level C3 activation may be partly responsible for low-grade redness during lens wear and also for certain discomfort sensations such as itchiness.

65

CL WEAR REDUCES PMN RECRUITMENT INTO TEARS

Stapleton *et al*., 1997

Sampling occasion	Non-lens wear median	Lens wear median
Open Eye	0	0
8 h Sleep	2777	181

N = 6

98650-60S.PPT

6L598650-60

Polymorphonuclear Leukocyte Recruitment

A study by Stapleton *et al.* (1997) investigated the effect of contact lens wear on the recruitment of PMNs.

The subjects wore ionic hydrogel lenses (FDA Group 4) on a daily wear basis and then had one night of extended wear.

The results show a significant difference in the number of PMNs present in the tears following overnight wear. This possibly predisposes the anterior eye towards colonization by bacteria during sleep in contact lenses, and therefore increases the chance of ocular infections or inflammation.

Because of the lack of an effect of lens wear on cytokines and arachidonic acid metabolites, it appears that reduced numbers of PMNs may be simply the result of the physical presence of the contact lens.

66

EFFECT OF CL WEAR ON LEVELS OF sIgA IN TEARS

sIgA: ***% of total protein***

Tear type	No lens	DW	EW
Closed Eye	54	-	51
Open Eye	22	13	10

N = 6

98650-61S.PPT

6L598650-61

67

EFFECT OF REDUCED sIgA IN TEARS

- Micro-organisms would be more likely to adhere to surfaces in the eye and grow
- Toxins would be less readily neutralized
- PMNs would be less able to phagocytose

98650-62S.PPT

6L598650-62

Level of Secretory IgA in Tears

The main immunoglobulin in the tears, sIgA, is significantly reduced during open eye contact lens wear (Pearce *et al.*, 1999) when compared with no lens wear.

Extended contact lens wear (EW) causes a significantly greater reduction in sIgA than does daily lens wear (DW). The level of sIgA in the tears on eye opening after sleep is not affected by contact lens wear.

The reduction in sIgA may enable micro-organisms, or their toxins, to cause inflammatory reactions in the eye.

68

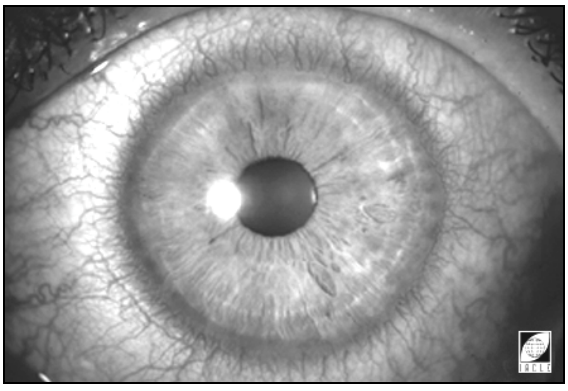
THE EFFECT OF CL WEAR ON HOST DEFENCES

- Alters normal functions of the epithelium (Dk/*t* dependent)
- Reduces PMN numbers (lens type dependent?)
- Decreased sIgA

98650-63S.PPT

6L598650-63

69



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Contact Lens Wear and Ocular Defence Mechanisms

Contact lens wear does **not**:

- Alter lysozyme, lactoferrin or mucin concentrations.
- Cause large amounts of complement activation.
- Affect cytokine and arachidonic acid metabolite levels.

There is no evidence that contact lens wearers who experience an adverse response such as a Contact Lens-induced Acute Red Eye (CLARE) (slide 69) during lens wear have any absolute deficiencies in host defence systems.

Many adverse responses are the result of bacterial colonization of lenses and subsequent host inflammation.

70

INFLAMMATORY COMPONENTS IN CLARE

Inflammatory component	Reflex tears mean/ml	CLARE tears mean/ml
C3	4.0µg	4.3µg
Factor B	0.1µg	0.3µg
IL-6	75pg	116pg
IL-8	0.5ng	2.7ng*
LTB_4	250pg	636pg*

N = 6
*p < 0.05

98650-64S.PPT

6L598650-64

71

EFFECT OF CLARE ON LEVELS OF PMN IN TEARS

Holden *et al.*, 1996

	CLARE median	NORMAL median
PMN numbers	4	4
Epithelial cell numbers	28	15

N = 12

98650-65S.PPT

6L598650-65

Inflammatory Components in CLARE

During a CLARE there are increased levels of IL-8 and LTB_4 in the tears (Thakur and Willcox, 1998). Presumably these substances chemo-attract PMNs into the tear film and stroma, producing the corneal infiltrates associated with this condition.

There is no evidence of complement activation during a CLARE. Nor are there significant differences in the numbers of PMNs or epithelial cells in the tears. This is probably due to the high level of reflex tearing associated with this condition.

However, a large increase in the number of PMNs seen in the corneal stroma occurs during the CLARE event.

72

INFLAMMATORY COMPONENTS IN CLPU

Inflammatory component	Reflex tears mean/ml	CLPU tears mean/ml
C3	4.0µg	3.7µg
Factor B	0.1µg	0.1µg
IL-6	75pg	62pg
IL-8	0.5ng	0.8ng
LTB_4	250pg	1271pg*

N = 8
*p < 0.05

98650-66S.PPT

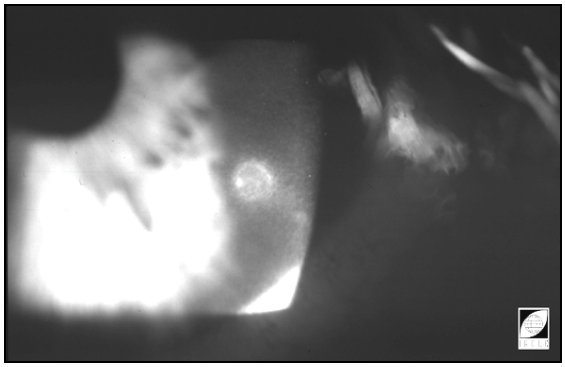
6L598650-66

Inflammatory Components in CLPU

In a Contact Lens-induced Peripheral Ulcer (CLPU) adverse event (slide 73) there are increased levels of LTB_4, indicating that this is the major chemo-attractant during this type of acute inflammation (Thakur and Willcox, 1998).

There is no evidence for an activation of complement or an increase in interleukins in CLPU.

73



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74

TEAR IMMUNOGLOBULIN LEVELS IN GPC

Immunoglobulin	GPC:Normal	% Plasma Protein
IgA	1	–
IgG	2	↑
IgM	>5	↑
IgE	3	↑↑↑

Donshik *et al*., 1983; Barishak *et al*., 1984

98650-68S.PPT

6L598650-68

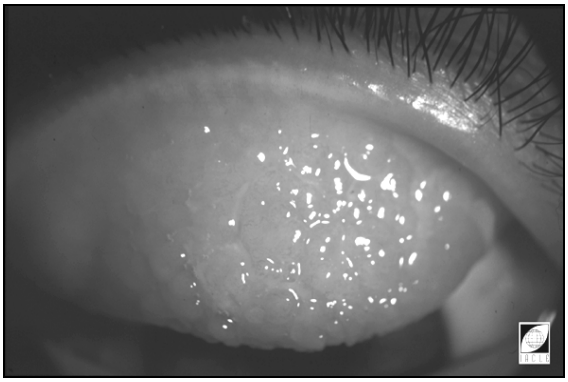
Tear Immunoglobulin Levels in Giant Papillary Conjunctivitis

Giant papillary conjunctivitis (GPC) (slide 75) has features of both a Type I response, i.e. an IgE-mediated hypersensitivity, and a Type IV delayed reaction.

The Type I (immediate hypersensitivity) response is mediated by specific IgE associated with mast cells in the conjunctiva. However, the nature of the specific antigen(s) has not been clearly established.

The delayed inflammatory reaction is mediated by sensitized lymphocytes reacting with an antigen to release lymphokines, with resultant tissue inflammation and damage.

75



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76

CHANGES IN NON-SPECIFIC TEAR DEFENCE PROTEINS IN GPC

Protein	GPC:Normal	% Plasma Protein
Lactoferrin	0.6	–
Lysozyme	1.1	–
C3	3.8	↑↑↑
Factor B	3.5	↑↑↑

Ballow *et al*., 1987; Rapacz *et al*., 1988; Ballow *et al*., 1985

98650-69S.PPT

6L598650-69

Non-specific Tear Defence Proteins in GPC

Studies have shown that the concentration of lactoferrin in the tear secretion of patients with GPC decreases slightly, while the concentration of lysozyme remains unchanged.

A significant increase in the concentration of complement protein C3 and Factor B, which regulates complement activation, has been demonstrated.

77

WHITE BLOOD CELLS SEEN IN CONJUNCTIVA DURING GPC

- Increased:
  - granulocytes
  - mast cells
  - eosinophils
  - basophils

Allansmith *et al*., 1977

98650-70S.PPT

6L598650-70

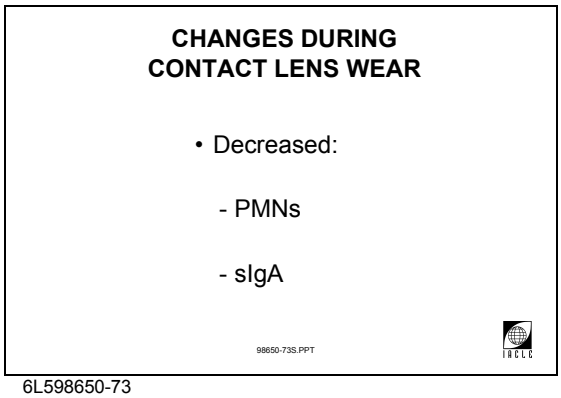
White Blood Cells and GPC

Cellular infiltration of the conjunctival epithelium with mast cells, eosinophils and basophils, etc. is regularly observed in GPC.

The presence of infiltrates in abnormal locations in the conjunctival tissue reflects the disturbed nature of the immune apparatus in this condition.

IV Summary

78



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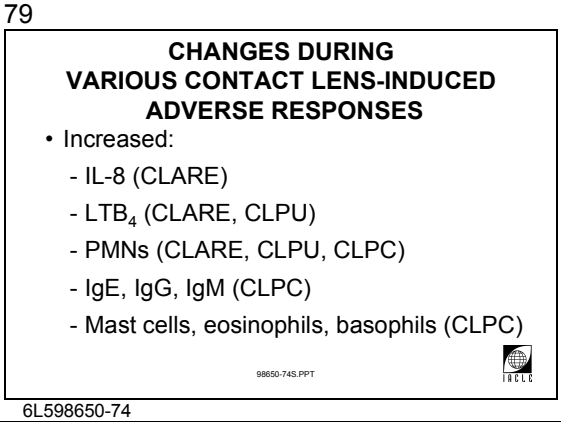
Summary: Changes in Contact Lens Wear

79



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Summary: Changes in Contact Lens-Induced Adverse Responses

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