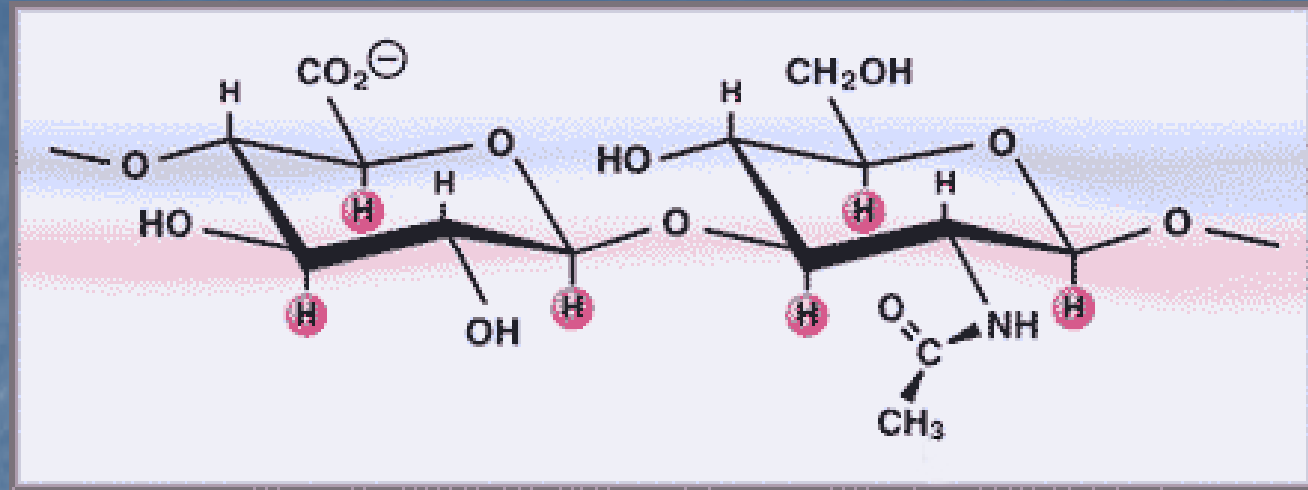
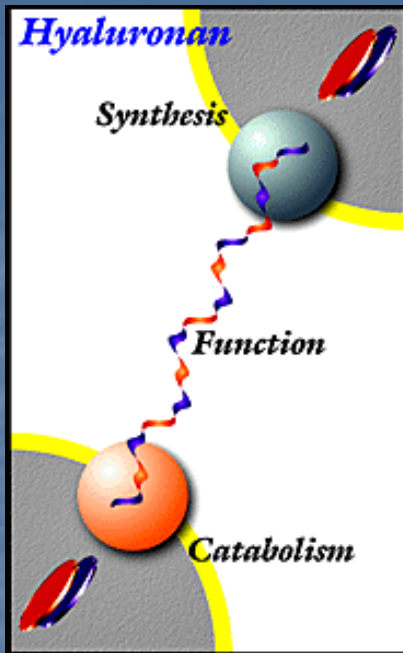


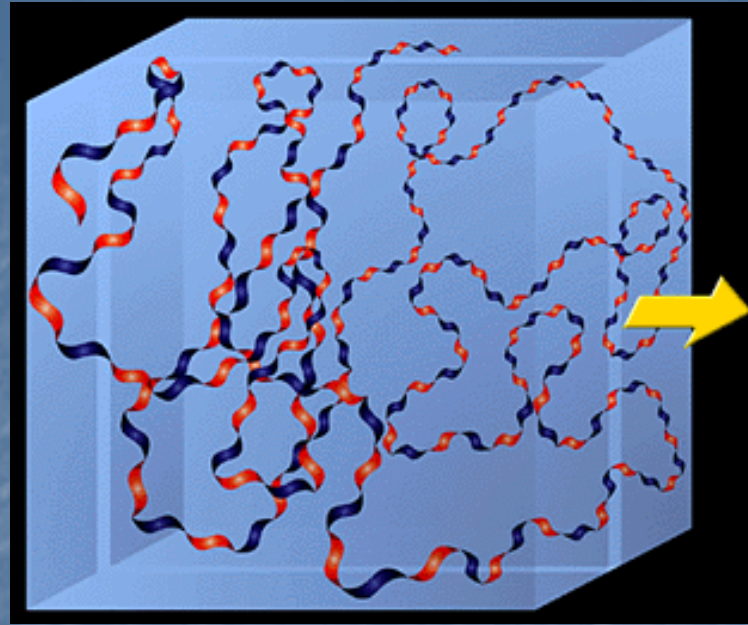
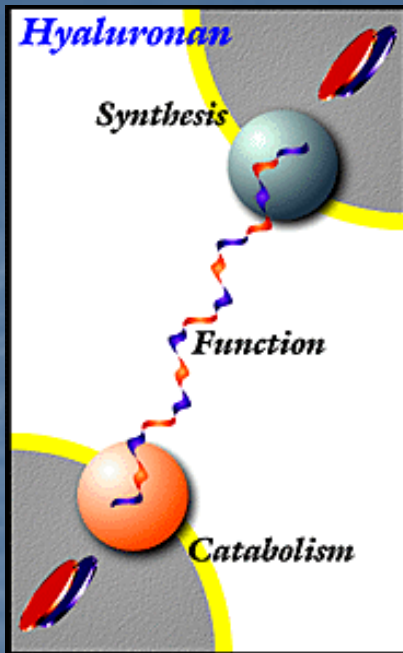
# Everything You Wanted to Know About Hyaluronic Acid

## I. Hyaluronic acid

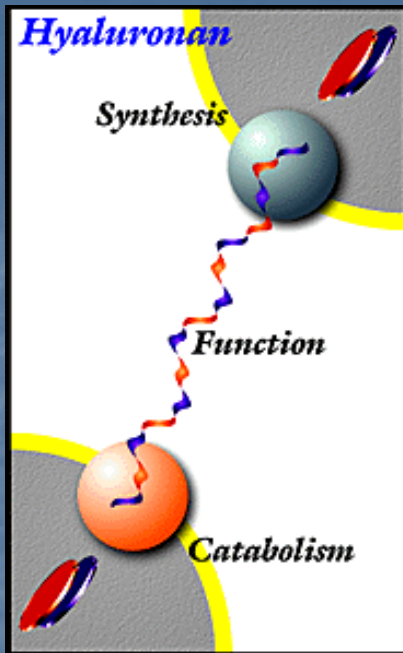
Presented by  
Dr. Klaus Laeschke



Hyaluronic acid ( HA ), also calling Hyaluronan, is a naturally occurring polysaccharide consisting of linear chains of alternating D-glucuronic acid and N-acetyl-D-glucosamine residues that forms an important structural element in the skin and connective tissues.



The domain structure of hyaluronan has interesting and important consequences. Small molecules such as water, electrolytes and nutrients can freely diffuse through the solvent within the domain. However, large molecules such as proteins will be partially excluded from the domain because of their hydrodynamic sizes in solution. As shown in the Figure, the hyaluronan network in the domain allows less and less space for other molecules the larger they are.



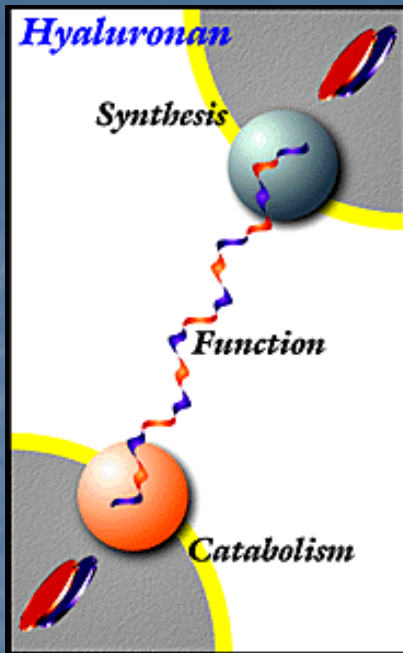
In a normal human body ( 70 kg ) there is about 15 g of HA.

Half of this mass ( 56% ) is contained in the human skin ( 7-8 g ).

A highly content of HA is in the basal membrane and the papillary dermis.

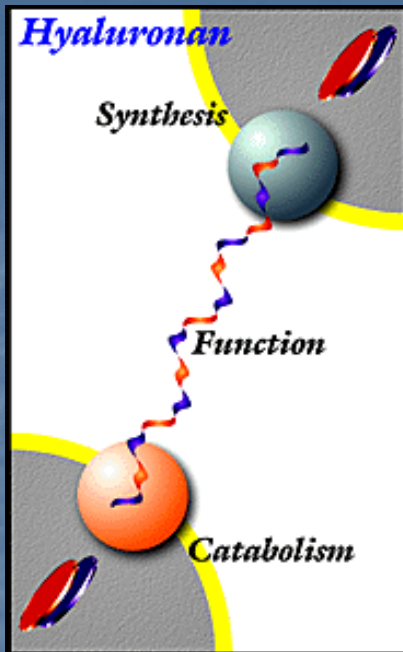
The concentration of HA goes down in the reticular dermis.

The absolute content of HA in the epidermal cellular matrix is with 2-4 mg/ml 10 times higher than in the dermis.



HA is strongly hydrophilic and performs a natural hydrating function within the dermis and epidermis, promoting skin suppleness.

The HA is not only a structure former. It regulated also the cellular process of growth.



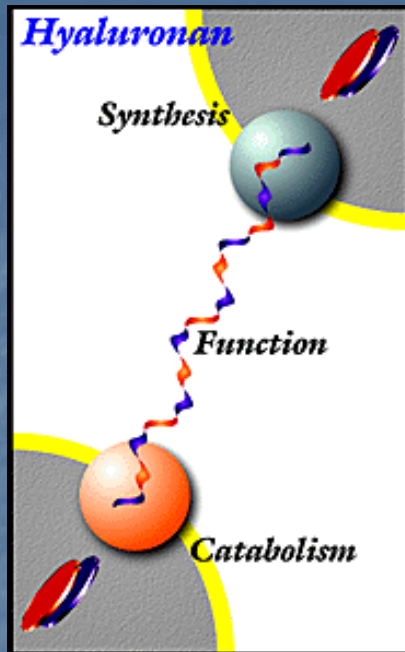
Predominant component of the extracellular matrix in the epidermis.

An effective scavenger of free radicals.

HA remove and clear noxious compounds from the epidermis.

Cells of the immune system move in and out of the epidermis. HA facilitate the movement of these cells.

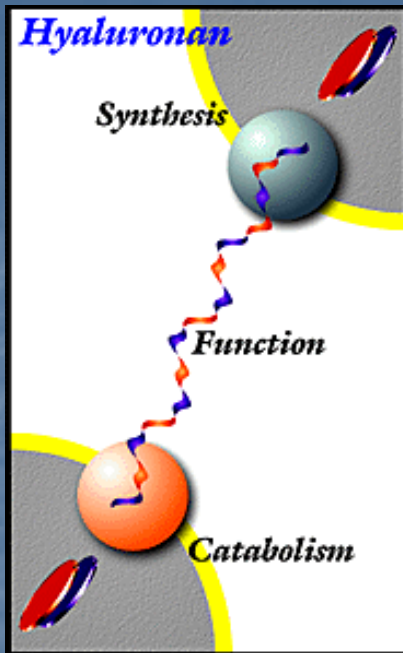
HA enhance the constant epidermal cellular remodeling



HA is responsible for the motility of the cells, regulation of the growth and differentiation, and the wound healing process by binding to cytokines.

In many cases the hyaluronan is organized into the extracellular matrix by specific interactions with other matrix macromolecules.

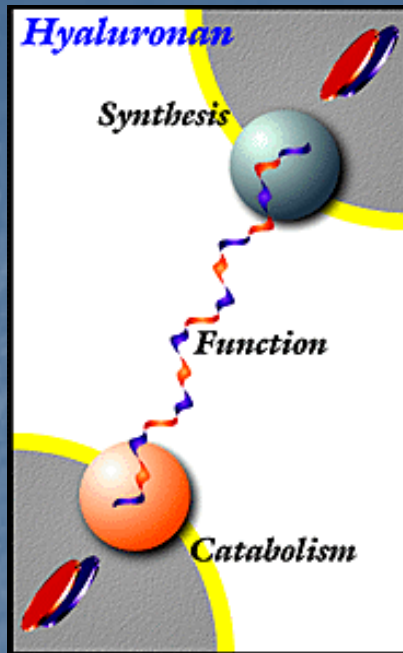
HA has a visco-elastic nature while preserving a high level of hydration ( water content 99% ).



HA is synthesized through the enzymes Hyaluronic-Acid-Synthase ( HAS ).

Synthesis is usually balanced by catabolism, thereby maintaining a constant concentration in the tissue.

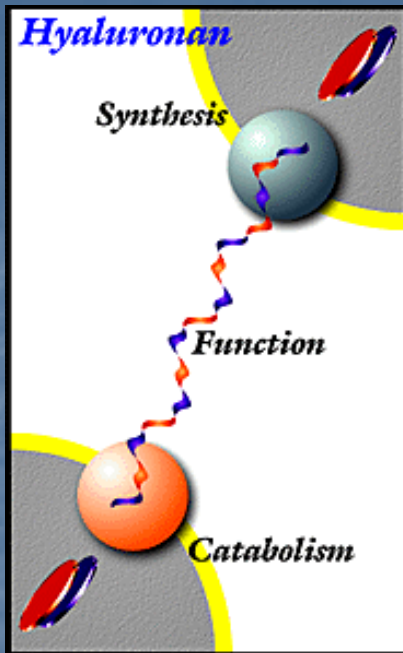
Keratinocytes in epidermis are another example of cells that actively synthesize and catabolize hyaluronan. In this case, the half life of a hyaluronan molecule is surprisingly short, less than a day.



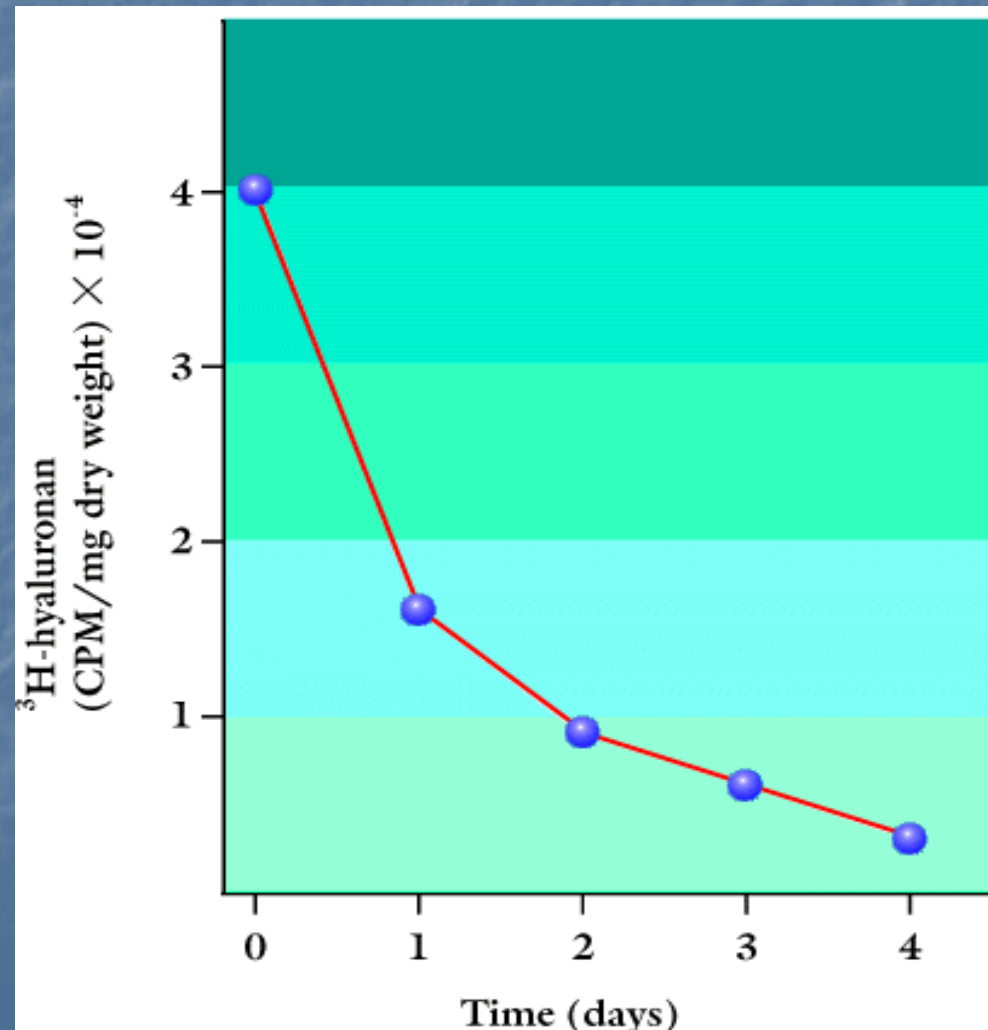
Sometimes cells either predominantly synthesize or they catabolize hyaluronan. For example the cells in the dermis actively synthesize more hyaluronan than they catabolize.

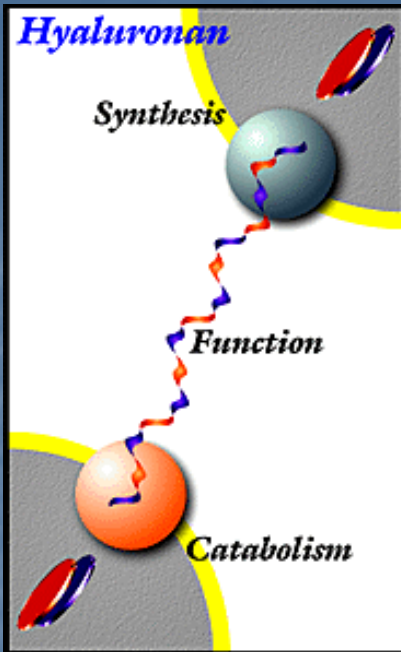
The half life of a hyaluronan molecule in the blood is very short, only a few minutes. Tissues in joints, such as the lining cells of the joint capsule of the knee, synthesize hyaluronan and release it into the synovial fluid, where it becomes a major component that contributes to the viscoelastic properties of the fluid.

It has been estimated that almost one-third of the total hyaluronan in the human body is metabolically removed and replaced during an average day.



The metabolism of hyaluronan in the epidermis. Explants of adult human skin were incubated for 24 h with [<sup>3</sup>H]glucosamine.

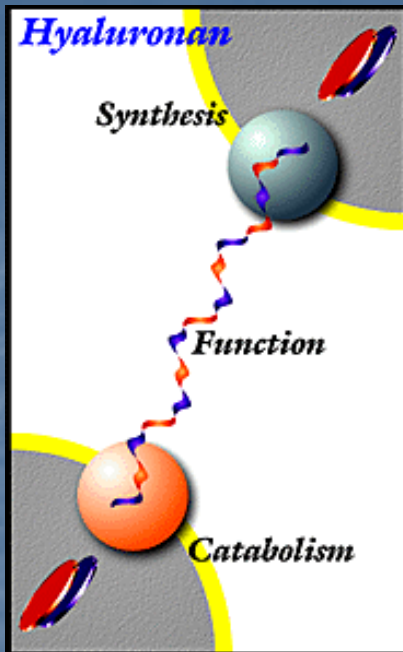




# Everything You Wanted to Know About Hyaluronic Acid

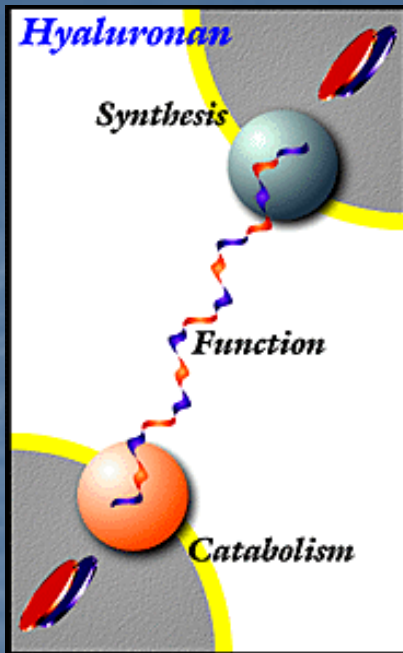
## II. Industrial Production of HA

Presented by  
Dr. Klaus Laeschke



HA can be obtained from two different sources:

- Animal
  - harvested from rooster combs
- Non-animal
  - Produced by fermentation
    - Streptococcus equi
    - Bacillus subtilis



# HA can be obtained from two different sources:

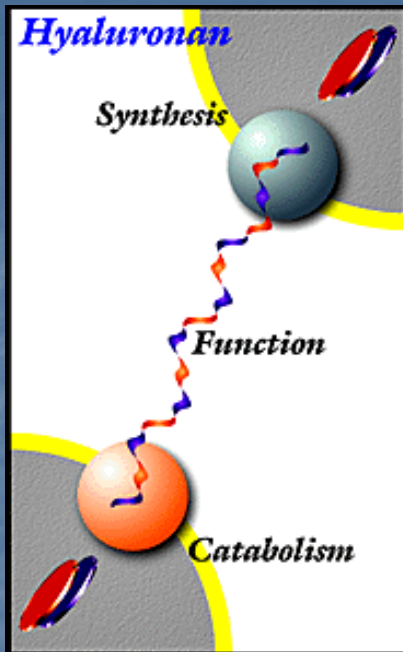
- Animal ( by extraction )
  - Advantage: high MW possible ( up to 5 Mill Dalton )
  - Disadvantage: protein content, risk for allergic reactions.
- Non-animal ( by fermentation )

## Streptococcus equi Lancefield A and C

- Advantage: No foreign body reactions, Taylor made MW from 50.000 to 2.5 Mill Dalton
- Disadvantage: endotoxin content, immunologic reactions.

## Bacillus subtilis

- Advantage: Endotoxin free
- Disadvantage: No clinical data for injectables.

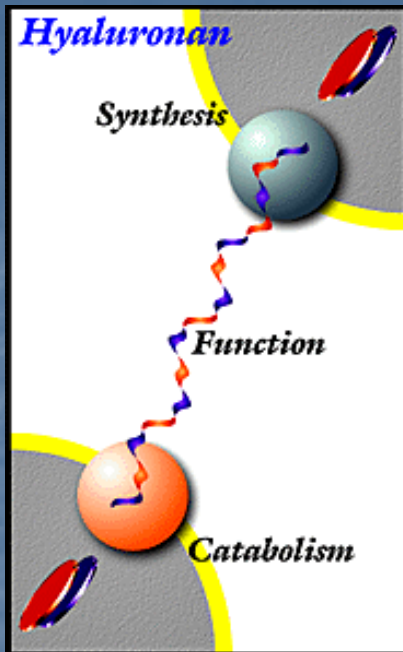


## Regulatory requirements

Basic information given by the European Pharmacopoeia.

01/2005:1472 Sodium Hyaluronate

HA is produced by methods of manufacture designed to minimise or eliminate infectious agents. When produced by fermentation of gram-positive bacteria, the process must be shown to reduce or eliminate pyrogenic or inflammatory components of the cell wall.



## Regulatory requirements

Basic information given by the European Pharmacopoeia.

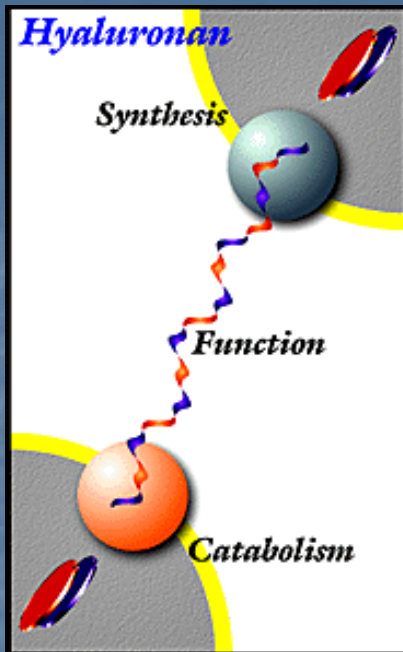
01/2005:1472 Sodium Hyaluronate

*Microbial contamination ( animal source ):*

Total aerobic viable count not more than  $10^2$  micro-organisms per gram.

*Bacterial endotoxins ( Streptococci ):*

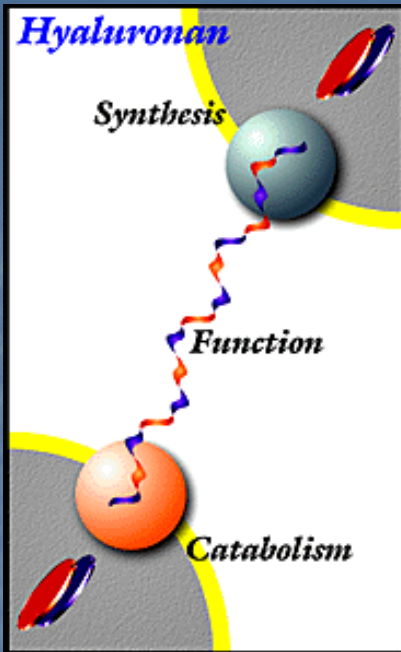
Intended use intra-ocular or intra-articular removal of bacterial endotoxins, less than 0.05 IU/mg.



# CONCLUSION

The purity of the HA depends on the origin and extraction/isolation method.

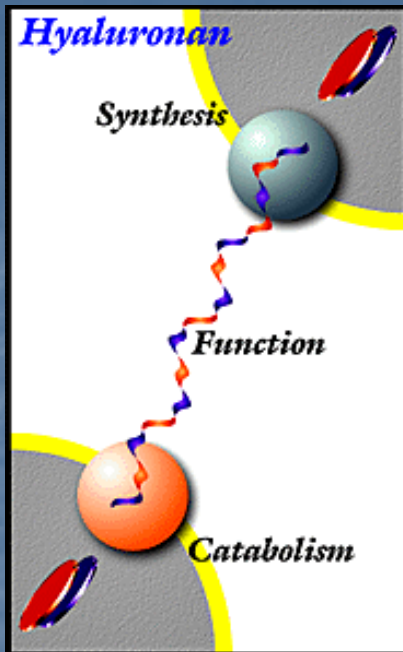
Specially the content of proteins and endotoxins are important to avoid side effects after implantation in the tissue or joints.



# Everything You Wanted to Know About Hyaluronic Acid

## III. Reticulation. Cross-linking

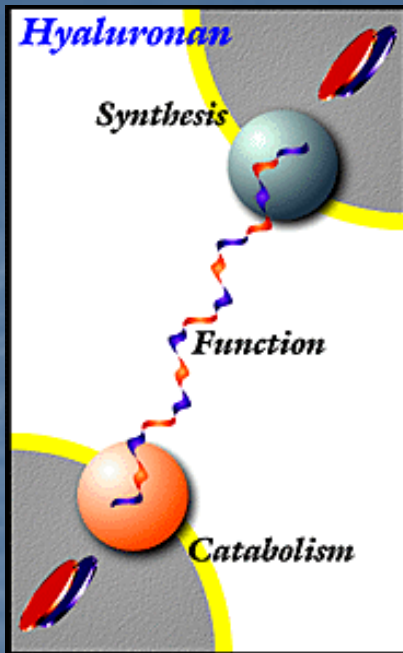
Presented by  
Dr. Klaus Laeschke



Because of the short duration in the tissue, the rapid tissue turnover (24 hrs) make the non-modified HA unsuitable for use as an exogenous soft tissue filler.

Chemical cross-linking/reticulation of HA results in the formation of an water-insoluble visco-elastic polymer with improved resistance to enzymatic degradation.

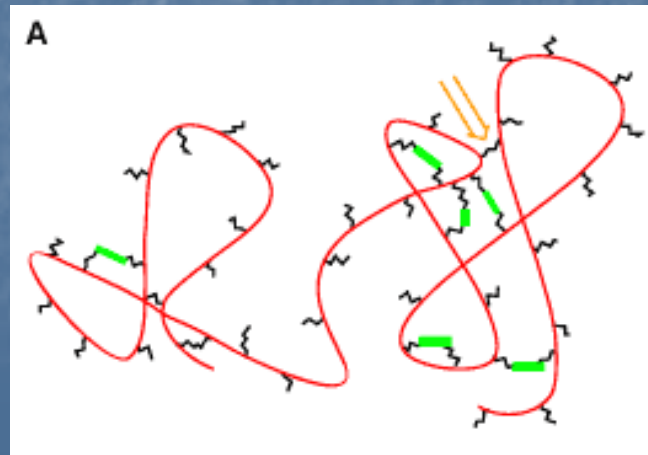
The chemical name of crosslinked HA is HYLAN GEL ( Balazs 1989 ).

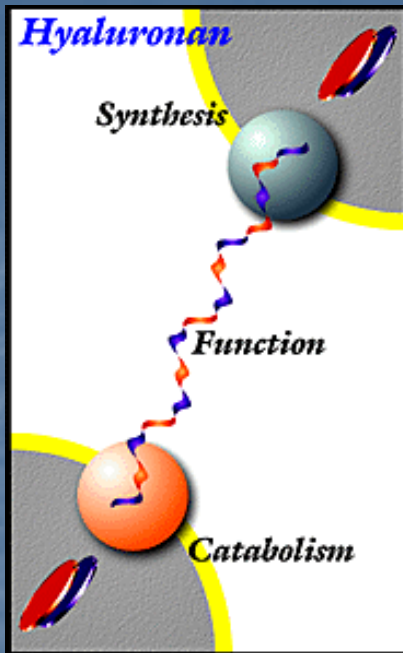


Hyaluronan (HA), an abundant non-sulfated glycosaminoglycan component of synovial fluid and extracellular matrices, is an attractive building block for new biocompatible and biodegradable polymers that have applications in drug delivery, tissue engineering, and viscosupplementation.

A variety of chemical modifications of native hyaluronan have been devised to provide mechanically and chemically robust materials. The resulting hyaluronan derivatives have physicochemical properties that may significantly differ from the native polymer, but most derivatives retain the biocompatibility and biodegradability.

The prototypical modification is conversion of the viscous sol form to a cross-linked hydrogel ( Fig. A ).



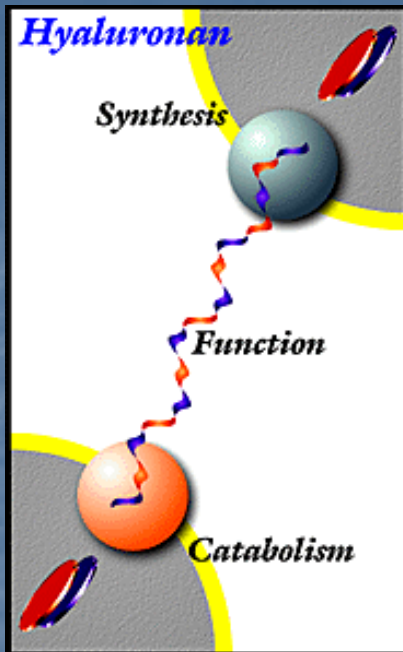


There are three different chemical substances accepted for cross-linking:

DVS ( Divinylsulfon )

BDDE ( 1,4-butanediol-diglycidyl-ether)

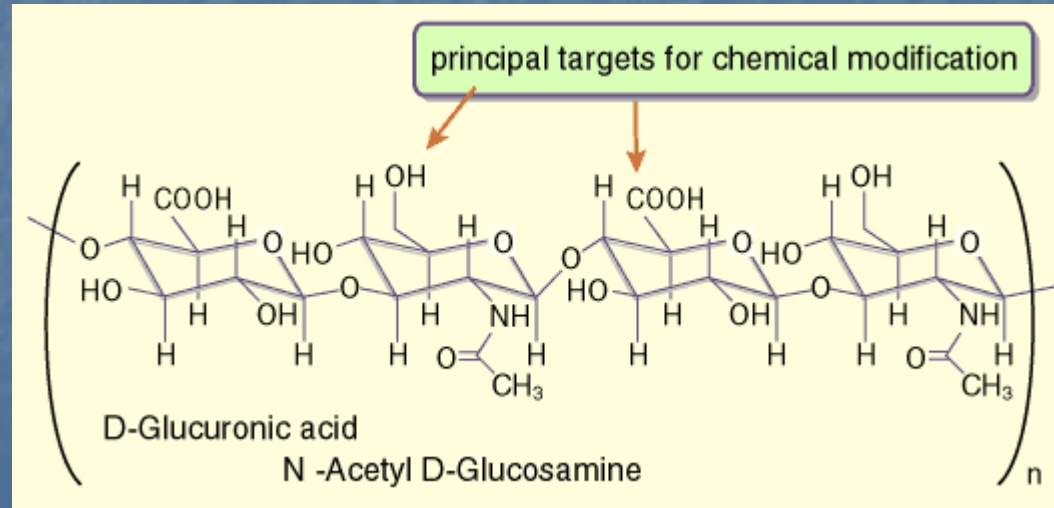
DMF (Dimethylformamide)

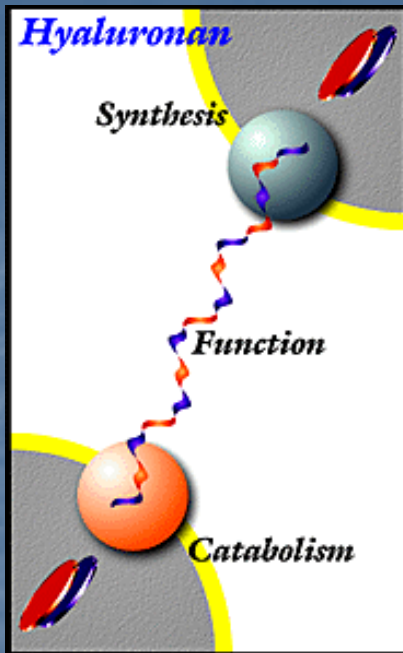


## Crosslinking/Reticulation

- Reactions of carboxyl groups
- Reactions with hydroxyl groups

This figure shows the chemical structure of hyaluronan with the two most commonly used sites of covalent modification: the carboxylic acid and hydroxyl functionalities.

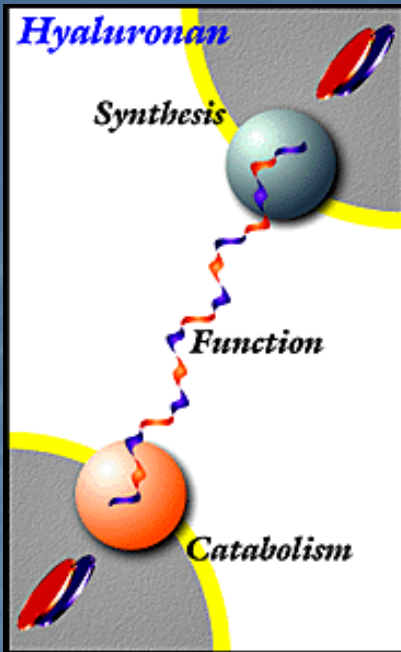




# Essential requirements

Hylan Gel is water-insoluble, but it binds water while injected into the soft tissue. For that reason it is important to saturate the Hylan gel during the process of manufacturing with non-crosslinked HA, as a water reservoir, before release the Hylan Gel as a soft tissue filler.

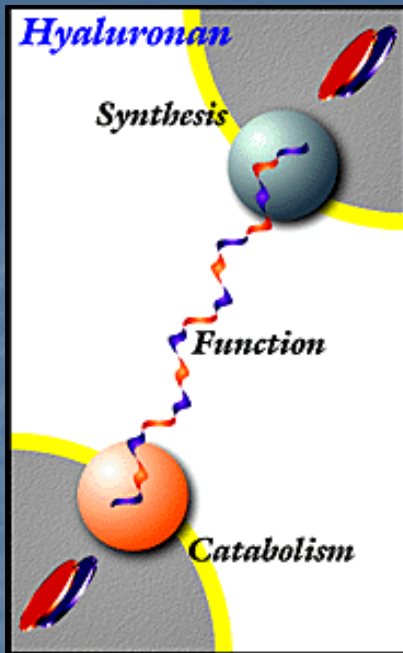
An important issue after the crosslinking process is the determination of the non-reacted crosslinker, because all of the crosslinker are cytotoxic. For that reason it is necessary, the the non-reacted crosslinker has to be washed out.



# Everything You Wanted to Know About Hyaluronic Acid

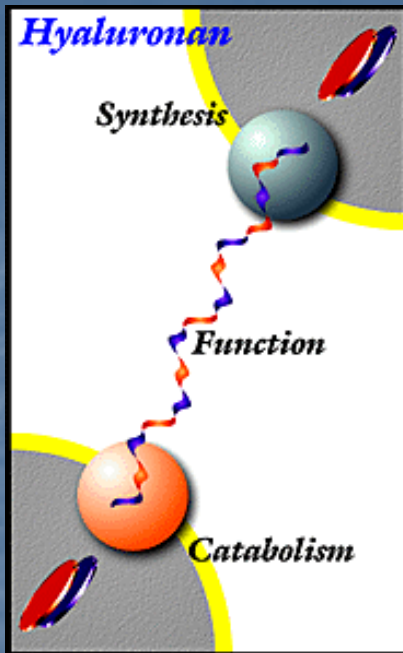
## IV. Effectiveness and Risks

Presented by  
Dr. Klaus Laeschke



In the last decade, Hyaluronic acid has been shown to possess many properties that suggest its value in several medical applications:

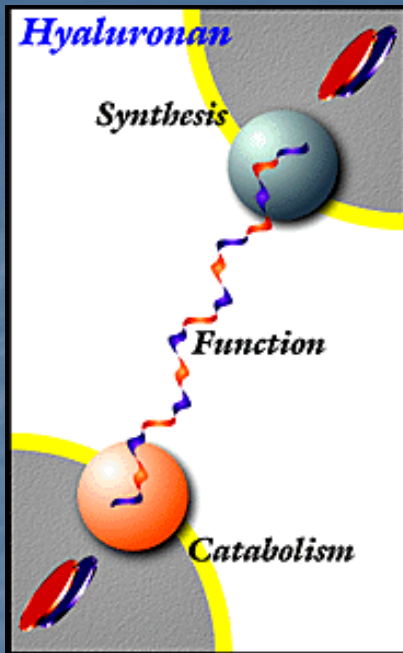
- Ophthalmology
- Orthopedics
- Urology
- Soft tissue augmentation
- Wound healing
- Delivery system for drugs



The effectiveness of HA is based on the biocompatibility and the visco-elastic property.

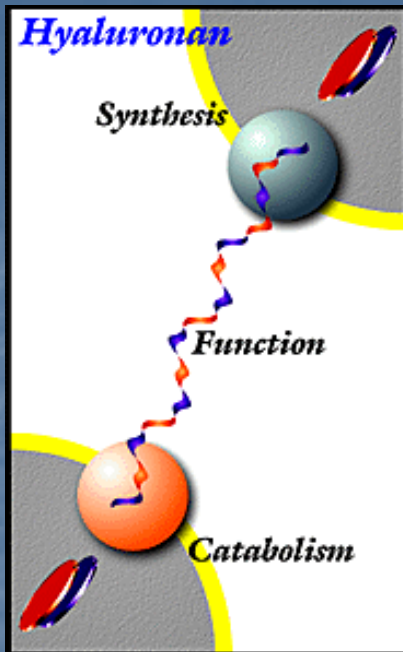
Non modified HA does not have adequate resistance to degradation.

To use as a soft tissue augmentation material it is necessary to crosslink the HA in Hylan Gel.



The crosslinked HA retained the biocompatibility and biologic properties like the non- modified substance.

The infinite network of cross-linked polysaccharide chains provides a material with superior residence time in dermal tissue.



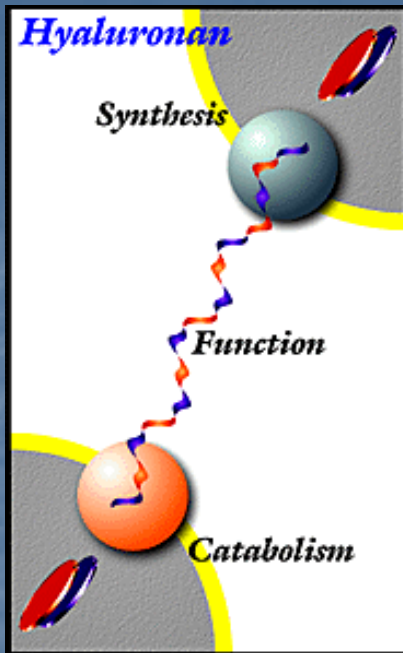
## Risks with Hylan Gel

Since 2000 we find clinical papers, showing that the Hylan Gel can create hypersensitivity reactions.

**Lowe:** Hyaluronic acid skin fillers. Adverse reaction and skin testing  
J Am Acad Derm. 2001;45:930-933

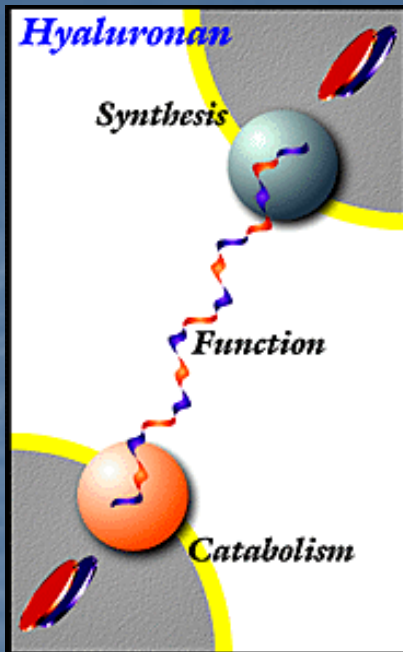
**Brody:** Use of Hyaluronidase in the treatment of granulomatous hyaluronic acid reactions.  
Derm Surg 2005;31:893-897

**Lupton:** Cutaneous hypersensitivity reaction to injectable hyaluronic acid gel.  
Derm Surg 2000;26:135-137



The reaction described are

- Allergy to the material
- Immunologic response to protein contaminants in the HA preparation
- Misplacement of the material

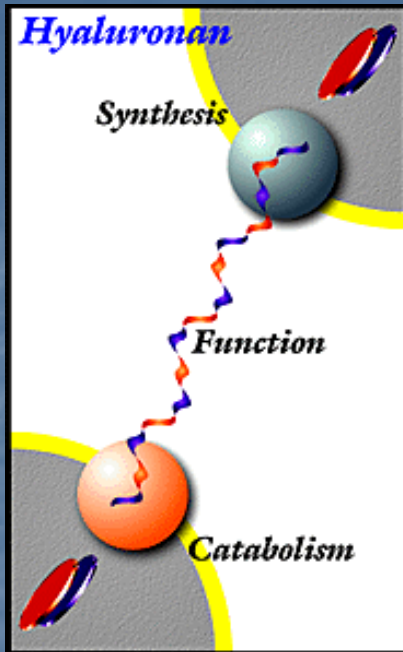


How to treat this adverse reactions?

**Hyaluronidase** ( derived from bovine testicular tissue)

This enzyme is the first choice

Hyaluronidase is a soluble protein enzyme that acts at the site of local injection to break down and hydrolyze hyaluronic acid.



Thank You  
for Your  
Attention

Dr. Klaus Laeschke