Micronisation of Collagen

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Objectives

- Isolation and purification of different types of fibrillar collagen
- Chemical, physical and enzymatic modifications of collagen
- Major objective is to stabilize collagen against degradation *in vivo*
- Formulation of collagen preparations
- Testing of anti-inflammatory and cytotoxic properties in cell cultures *in vivo*

Isolation of Collagen

- Primary calf skin is used in the beginning of the project; it is planned to isolate collagen from other sources such as fish skin
- To minimize fluctuations of the chemical and biological quality of collagen, only acid-soluble collagen (ASC), pepsin-soluble collagen (PSC) and alkali-treated collagen are used

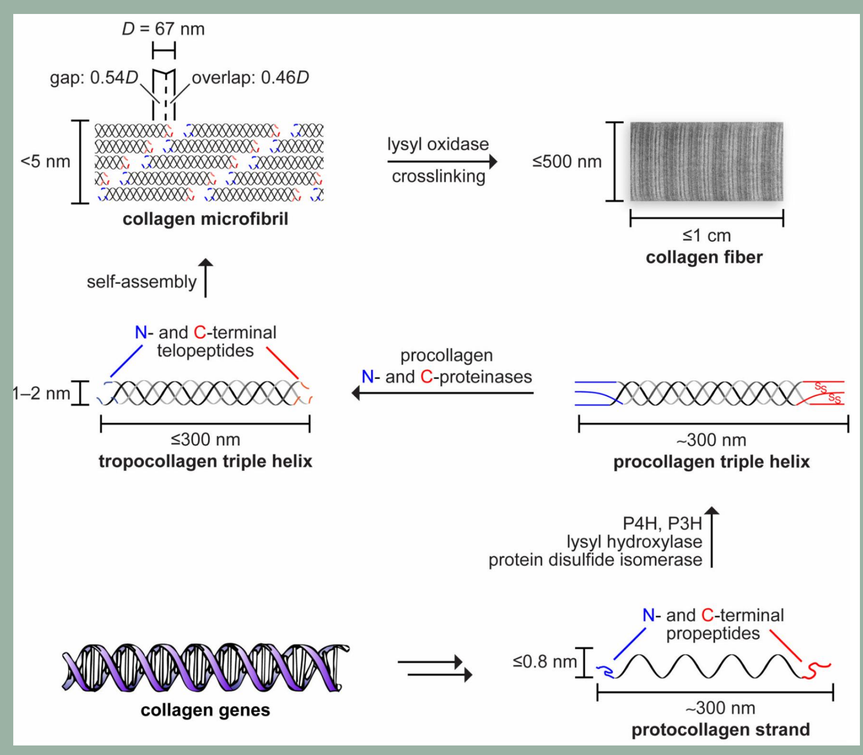

Figure 1: Biosynthetic route to collagen fibers [1]

Physical Modifications

- Under UV-light (254 nm) the aromatic rings of phenylalanine and tyrosine form free radicals and react with each other. The crosslink formation only takes place on the surface of collagen
- For dehydrothermal treatment (DHT) the collagen sample is heated up to <90°C while under vacuum. During water evaporation additionally crosslinks are formed through a condensation reaction between carboxyl and amino groups

Chemical Crosslinking Reagents

Two different methods are common:

1. Linker between two free amino groups of the collagen triple-helix
    - Glutaraldehyde
    - Hexamethylene diisocyanate (HDI)

    Glutaraldehyde is the most commonly used reagent in literature, but release of cytotoxic products after degradation of collagen *in vivo* can occur

2. Formation of amide via reaction of a carboxylic acid with an amine
    - Acyl-Azide-Method
    - Carbodiimide Method like Ethyl-dimethyl-aminopropyl-carbodiimide (EDC) and N-Hydroxysuccinimid (NHS)

    The formation of an amide is preferable, because the reagents are not involved in the crosslink

Enzymatic Modifications

- Transglutaminase

  Catalyzes the chemical reaction between glutamine and lysine residues
- Oxidoreductase

  Oxidation of acid soluble collagen with e.g. tyrosinase or laccasein in combination with phenolic molecules

Analytics

Method	Information
SDS-PAGE	Size
CD spectroscopy	Secondary and tertiary structure
DSC	Denaturation temperature
SEM	Topography
Mass spectroscopy	Mass
Determination of free amino groups	Density of crosslink
Incubation with collagenases	Stability of collagen

Table 1: Overview analytical methods

Outlook

- Because collagen is isolated from natural products the variation in structure resp. composition is high. Isolation of a permanently invariable material is difficult and should be realised by ASC or PSC extraction
- Chemical, physical and enzymatic modifications have to be done to increase the stability of collagen against biological degradation *in vivo*
- Some analytical methods need to be established, most methods are well described in literature
- Many chemical, physical and enzymatic modifications are published; however the effect of modification on the chemical, physical and biological properties of collagen is not always predicable and should be determined

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