Easy quantification of collagen and protein in paraffin-embedded tissues or tissue sections

Natascha van Lent
Jessica Snabel
Reinout Stoop
Roeland Hanemaaijer
Jan Verheijen

TNO Metabolic Health Research
QuickZyme Biosciences
Jan.Verheijen@Quickzyme.nl
Roeland.Hanemaaijer@TNO.nl

TNO innovation for life

Introduction

Extraction efficiency of collagen from tissues is low and variable (table 1) probably due to the presence of crosslinks in mature collagen. Therefore a common way for quantitative analysis of collagen is acid hydrolysis of the tissue followed by measurement of hydroxyproline, either by HPLC or by colorimetric assays. This method is suitable for tissues and tissue extracts.

The major way in which tissues are stored is as paraffin embedded formalin fixed tissue. Until now histological staining followed by image analysis is the method to analyze paraffin-embedded tissue. Such methods do give qualitative information on localization of the collagen in the tissue, but are only semi-quantitative.

We have devised a method for quantitative analysis of collagen in formalin-fixed paraffin embedded tissues or tissue sections. Neither removal of paraffin with toxic solvents like xylene nor special equipment is required. The sensitivity of the method is such that it is applicable to quantify collagen using only a few 10 µm tissue sections.

Methods

See Fig.1 for a representation of the procedure. Five to ten 10 µm tissue slides (or a corresponding amount of fresh or formalin-fixed tissue) are transferred into a Sarstedt tube and upon addition of 150 µl 6M HCl hydrolyzed by o/n incubation at 95°C in a heat block.

Upon hydrolysis, without any pretreatment, 35 µl is used for collagen quantification using the QuickZyme total collagen assay (assay time 90 min) and 15 µl is used in the QuickZyme protein assay (assay time 30 min), which has specifically been developed for protein analysis in acid hydrolysates. The protein data are used as calibration for the unknown amount of tissue used for hydrolysis.

Results

Low and variable extraction efficiency of collagen from tissues

Tissue	% extracted
Lung	3
Kidney	10
Spleen	8
Heart	14
Dermis	9
Tendon	3

Table 1: *Extraction efficiency of collagen from various tissues*

Tissues were extracted in 0.5M acetic acid with pepsin, and solubilized collagen was determined using a Sirius Red based assay. The solubilized collagen was compared to total collagen in tissue, quantified after acid hydrolysis using a hydroxyproline assay

QuickZyme Biosciences

Assay principle

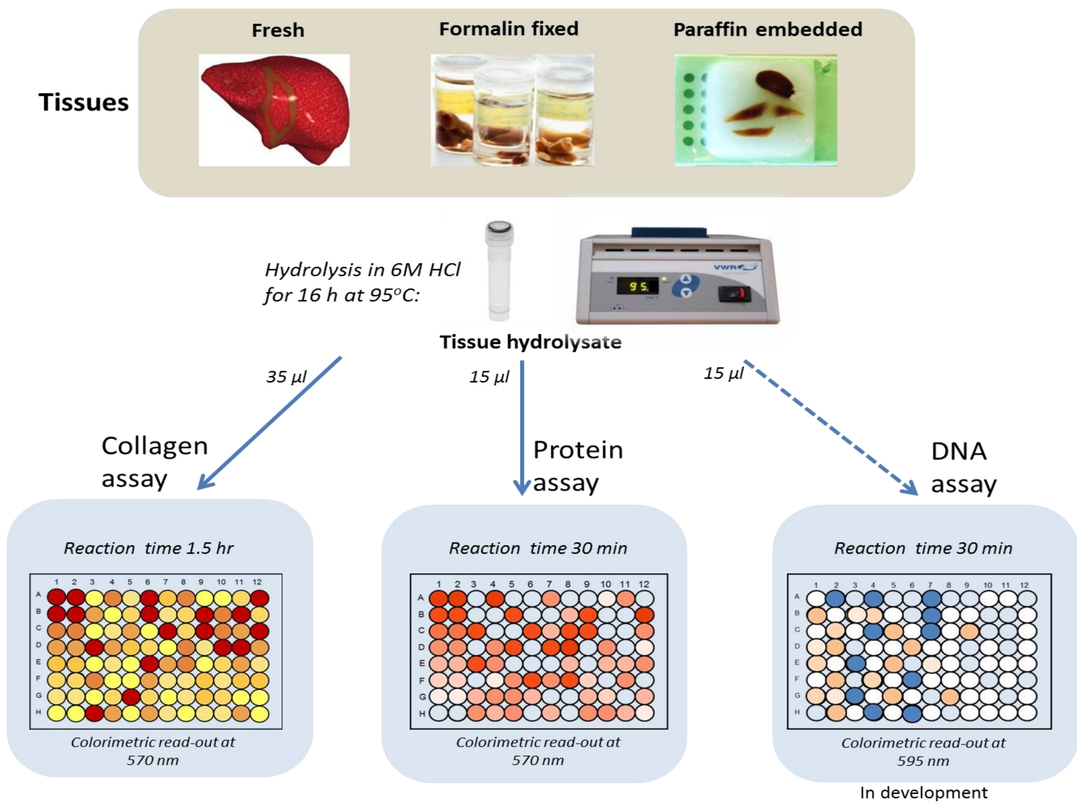

Figure 1: *Assay principle for quantification of collagen, protein and DNA in paraffin embedded formalin-fixed tissues*

Collagen and protein ratios are similar in frozen tissue, paraffinized and deparaffinized tissue sections

Treatment of samples, such as formaldehyde fixation, paraffin embedding and xylene treatment to deparaffinize the samples did not affect collagen and protein analyses (Fig 2).

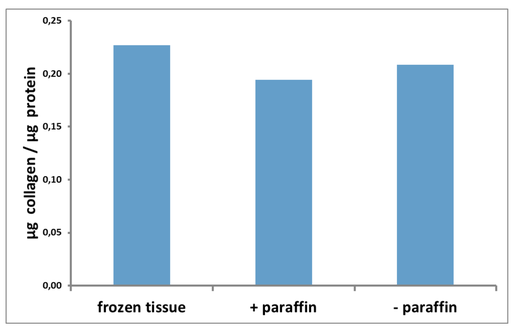

Figure 2: *Collagen/protein ratios in tissue processed in various ways*

Collagen was analyzed in formaldehyde-fixed paraffin-embedded human arterial tissue. Either frozen tissue, ten 10 µm paraffin embedded tissue sections or ten 10 µm tissue sections pre-treated with xylene to remove paraffin were hydrolyzed and analyzed using the QuickZyme total collagen assay and QuickZyme protein assay.

Collagen and protein analysis in sections from various types of (fibrotic) tissues

Starting from tissue sections, examples are given from mouse kidney fibrosis (UUO), lung fibrosis (bleomycin-induced), and liver fibrosis (NASH). Total collagen/total protein was measured from ten 10 µm paraffin embedded sections (Fig. 3A) , and was increased under fibrotic conditions. Also in human tissue sections (Fig. 3B) collagen/protein could be measured and was increased in aneurysm tissue.

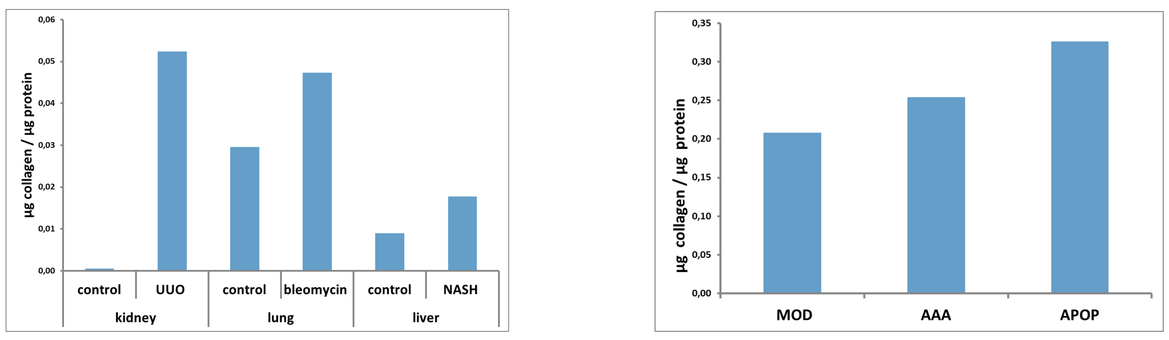

Figure 3: *Collagen/protein in various tissues*

Total collagen/total protein was analyzed in formaldehyde-fixed paraffin-embedded tissues from mouse kidney fibrosis (UUO vs Sham) lung fibrosis (bleomycin vs PBS) and diet-induced NASH (western diet plus cholesterol vs control) (Fig 3A), or human tissue from human abdominal aortic aneurysm (AAA), popliteal artery aneurysms (APOP) and controls (MOD)(Fig. 3B). Ten 10 µm tissue slides were hydrolyzed and analyzed using the QuickZyme total collagen assay and protein assay.

Conclusions

- **Quantification of collagen and total protein in formalin-fixed paraffin-embedded tissues is now possible in a fast and easy assay format, without the need for HPLC or other special equipment.**