A dot-blot based assay to quantify collagen from large arteries

Program LB201
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INTRODUCTION

Collagen is a protein which provides tissues with strenght and resistance to stretch and contributes to fibrosis in several diseases when it is synthesized in excess. Therefore collagen quantification is important and several methods are available, being colorimetric methods the most widely used:

1. **Hydroxyproline (HP) assay,** based on the detection of HP, an imino acid unique to collagen. The main disadvantage is that release of HP requires acid or alkaline hydrolysis and neutralization before the sample analysis, i.e. HP assay is timing consuming.

2. **Sircol Collagen assay (SCA),** based on the detection of Sirius Red (SR), which binds to collagen. The main disadvantage is the interference with albumin and the lack of detection of partially hydrolized samples.

Dot blot is a commonly used techinque to quantify small quantities of proteins bound on membranes. The **aim** of the present study was to develop a selective, sensitive, high-throughput and cheap densitometric assay based on Dot blot and SR staining able to quantify intact and partially hydrolyzed collagen in arteries.

METHODS

Experimental protocol

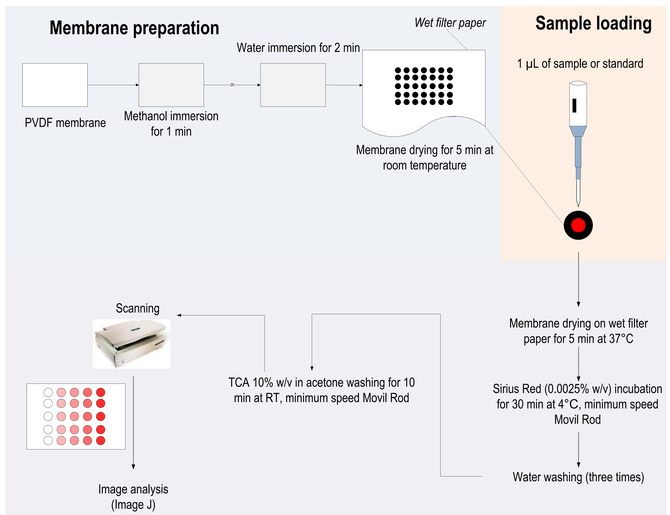
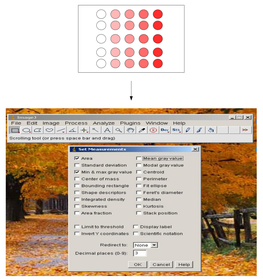


Image Analysis

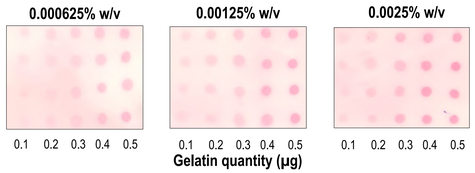


Densitometric parameter: integrated density (ID)

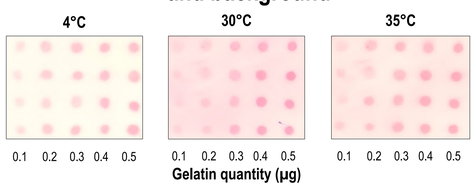
RESULTS

Optimization of a dot-blot based assay

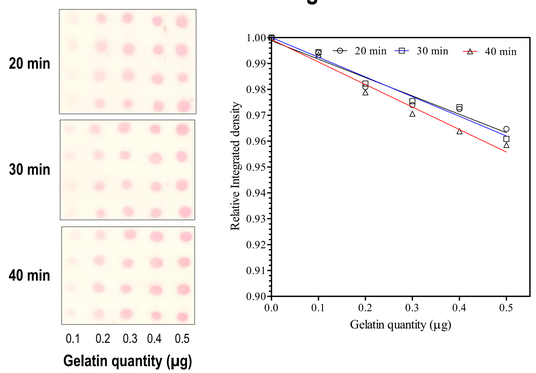
Effect of Sirius Red concentration on dot intensity and background



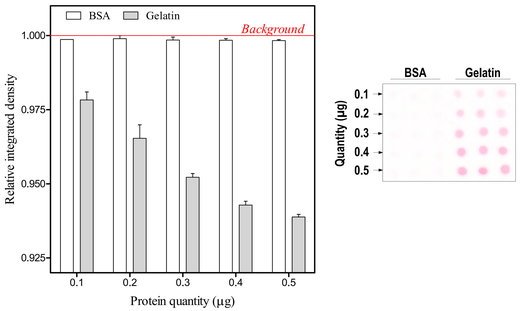
Effect of the incubation temperature on dot intensity and background



Effect of Sirius Red concentration on dot intensity and background

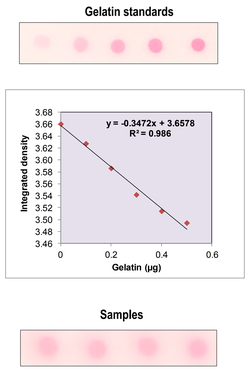


Interference of albumin with collagen quantification



Quantification of hydrolized collagen from rat carotid arteries

Sample	Mean integrated density	Gelatin (µg)	Collagen (mg gelatin/100 g dry weight)
1	3.516	0.4084	16.1
2	3.523	0.3882	15.4
3	3.528	0.3738	14.8
4	3.536	0.3508	13.9
5	3.533	0.3594	14.2
6	3.535	0.3537	12.0
7	3.542	0.3335	11.3
8	3.544	0.3278	11.1
9	3.524	0.3854	13.1
10	3.537	0.3479	11.8
11	3.553	0.3018	12.3
12	3.546	0.3220	13.2
13	3.558	0.2874	11.7
14	3.539	0.3422	14.0
15	3.555	0.2961	12.1
16	3.533	0.3594	11.3
17	3.525	0.3825	12.0
18	3.538	0.3450	10.8
19	3.524	0.3854	12.1
20	3.542	0.335	10.5



CONCLUSION

We have developed a quick, simple, cheap and specific method to quantify collagen in vascular samples compatible with elastin purification.

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