INTRODUCTION

Collagen is a protein which provides tissues with strength and resistance to stretch and contributes to fissing 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 technique 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 hydrolized collagen in arteries.

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

CONCLUSION

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