mination coefficient of R^2=0.83 (P=0.0309) was achieved, mostly with higher values in the histogram-analyzed results. This points to a higher sensitivity of the computer-based method over the planimetric analysis.

Discussion

The easy and fast quantification of fibrotic tissue is an important task in the histological analysis of cardiac remodeling in animal models. In the past several methods have been established to address this issue. A typical fibrosis staining method is the picrosirius red stain.[3] A couple of years ago, Gaspard and Pasumarthi[4] published a color-subtractive computer assisted image analysis (CS-CAIA)-based quantification method for diffuse fibrosis on sirius red/fast green stained tissue sections. However, this quantification approach requires a high color contrast, and is therefore poorly assignable to raw frozen tissue, which shows a reduced color contrast after picrosirius red staining compared to *e.g.* paraffin embedded sections, as shown in Figure 1.

In this study we present a new staining and histogram-based quantification approach for fibrotic tissue within the heart using FITC-labeled wheat germ agglutinin, which i) can be used for reliable quantification of scar formation after myocardial infarction in mice; ii) allows co-immunostaining with additional antibodies; and iii) is suitable for frozen tissue sections. WGA is a lectin originally derived from wheat, which binds to N-acetylglucosamin and sialic acid.[6,7] The fact that lectins can bind to carbohydrates, which are present on the cell surface, is known for more than 30 years. In combination with a histological label as horse radish peroxidase or fluorescent dyes, lectins are very suitable markers for cell membranes.[8-10] Next to the labeling of the glycocalix, it was also reported that WGA binds to connective tissue and extracellular matrix.[11-13] Components of the extracellular matrix, for example glycosaminoglycans as hyaluronic acid, are rich in N-acetylglucosamin, providing a rationale to use WGA for detection of scar tissue after myocardial infarction. The results of the present study clearly demonstrate that myocardial fibrotic tissue can be stained by WGA-FITC in cryosections. We found a correlation of R^2=0.96 between planimetric determined post-MI scar size after both, SR and WGA-FITC staining. This indicates that WGA-FITC is a suitable marker for fibrotic tissue. Furthermore, WGA-staining allows co-staining with *e.g.* anti-collagen I antibody for a structural differentiation of the extracellular matrix. Figure 4 demonstrates that parts of the scar are stained by either collagen I antibody or WGA in different intensities indicating a regional heterogeneity of different matrix components within the scar. With WGA/collagen I co-staining a differentiation and categorization of the extracellular matrix components can be performed.

In addition, we tested the ability to quantify the cardiac fibrosis amount with histograms of WGA-FITC and SR stained heart sections. Our results show that this histogram-based approach is suitable for the WGA-FITC label while the application on picrosirius red staining failed. A possible explanation might be the reduced color contrast due to elevated red background color of picrosirius red staining on cryosections. Nevertheless, WGA-FITC stains, differently from picrosirius red, also the cell membranes (Figure 4 B,C), leading to a background fluorescence, which has to be subtracted for the quantification of the fibrosis amount. Interestingly, we also found the histogram-based quantification of fibrosis to be more sensitive than the planimetric approach. The poor correlation between these methods applied to WGA-FITC stained sections (R^2=0.83) was due to higher fibrosis levels detected with the histogram-based approach. This higher sensitivity might be due to the fact that fibrosis also occurs apart from the substantial scar, *i.e.* in a diffuse form within the ischemic border zone. With the planimetry, it is not possible to cover this diffuse fibrosis and therefore this method rather underestimates the amount of fibrosis in post-MI hearts.

Taken together, there are several advantages for the use of the WGA-FITC staining- and quantification-approach as reported here, in comparison to other fibrosis staining and quantification methods. First, on raw frozen material common staining techniques like picrosirius red often do not show a high contrast between fibrotic and non-fibrotic tissue, as they do on paraffin-embedded tissue. However, this is a critical requirement for an automated quantification approach. On the contrary, WGA-FITC staining shows a high contrast and is therefore more suitable for this application. Second, the use of WGA-FITC allows the application of immunohistochemical staining with additional antibodies in the same approach, which is not possible in combination with histological preparations. Third, the use of WGA-FITC staining in combination with a histogram-based quantification is easy and fast to perform and therefore a competitive technique for the analysis of fibrosis amount on raw frozen tissue sections.

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