Supplementary data

Reproducibility of the gelatin layers prepared using "standard conditions" and chamber heights

11 gelatin layers prepared in independent experiments by using "standard conditions" were compared. A ROI was defined in the central region of each layer. The average thicknesses and the fluorescence intensities of each ROI are shown in Fig. S1. A good reproducibility of layer preparation is achieved, evidenced by the coefficient of variation of thickness and fluorescence intensity of 4.1% and 2.7%, respectively. The height of the chamber was measured by interferometry. The heights of sample chambers assembled by single and double layers of laminating adhesive were 26.6±0.4 µm (n=9) and 52.3±0.5 µm (n=9), respectively.
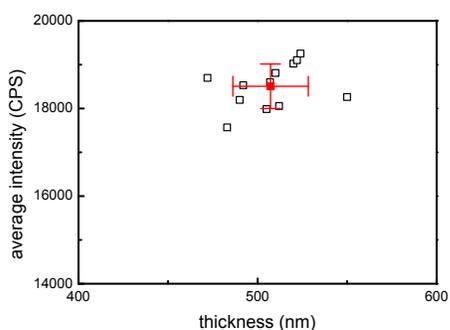

Figure S1: Thickness and fluorescence intensity in the central region of layers prepared using "standard conditions". Each black open square is the average result of a ROI in a specific layer. The red square with error bars represent mean ± standard deviations (n=11).

Influence of flow rates on release kinetics

0.25 µm and 1.5 µm thick layers were prepared and release experiments on these layers using different flow rates were performed. As shown in Fig. S2, the minor influence of the flow rate on the release kinetics of 0.25 µm and 1.5 µm thick layers in ~27 µm chambers suggests that perfusion plays a minor role in the release. Since 1.5 µm thick layers can swell to about 26 µm which is very close the height of the chamber with a single layer of laminating adhesive, the release kinetics of 1.5 µm thick layers in the chambers with different heights (~27 µm and ~52 µm) were compared. No significant differences of the release kinetics were observed. The fact, that the chamber height has hardly any influence on the release kinetics, indicates that the release takes place directly at the interface between the layer surface and eluting medium, even if there is only a very small volume of eluting medium left above the hydrogel layer.
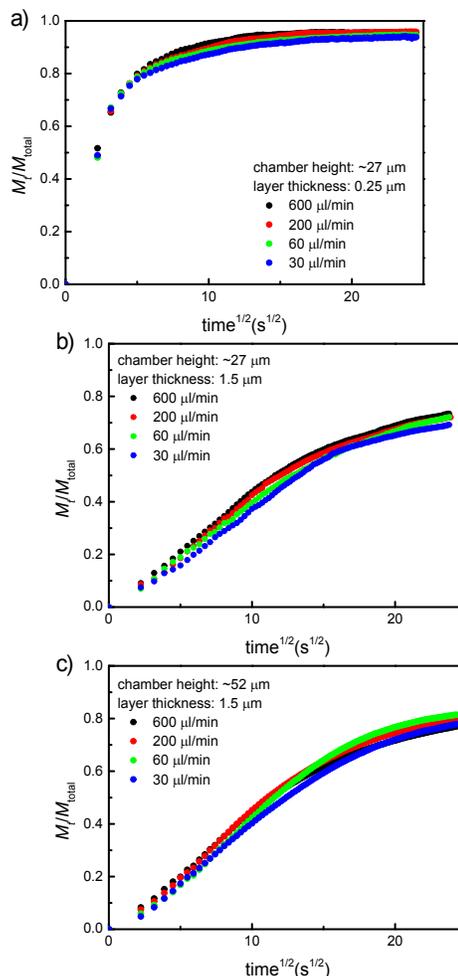

Figure S2: Influence of flow rates on release kinetics. a) 0.25 µm thick layer, chamber height: ~27 µm. b) 1.5 µm thick layer, chamber height: ~27 µm. c) 1.5 µm thick layer, chamber height: ~52 µm.

Statistical analysis of different release kinetics

To determine the significance of release time and diffusivity discussed in the manuscript, all the data were analyzed using ANOVA with subsequent Tukey test using SPSS 23. In Fig. S3a, decreased temperature and elevated humidity significantly increase the release time, while a significant reduction of release time is observed when the eluting medium is changed from MQ to PBS or plasma. Fig. S3b shows a significant inverse correlation between D and r. Although the results of normality test and homogeneity of variances test in SPSS show the samples within each condition group (temperature, RH, size of reagent, media) are normally distributed with equal variance, we remain cautious about the eligibility of the test and the significance claim, due to the limited sample size.
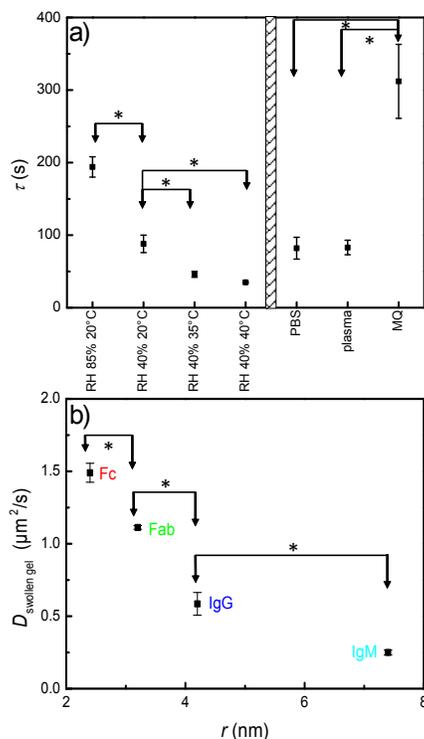
Figure S3: a) left: release times (τ) of IgG from gelatin layers prepared under different conditions in PBS, right: release times (τ) of IgG from gelatin layers prepared under "standard conditions" in different media. Data points represent mean ± standard deviation (n=3). The symbol (*) indicates a significant difference (p<0.05) in release time (τ) between the compared conditions. b) Diffusivity ($D_{swollen\ layer}$) of antibodies and antibody fragments in gelatin layers prepared under "standard conditions". Data points represent mean ± standard deviation (n=3). The symbol (*) indicates a significant difference (p<0.05) in diffusivity ($D_{swollen\ layer}$) between compared reagents.

Quantification of embedded IgG in gelatin layers

The stability of LED excitation and the minimized exposure to prevent photobleaching, allows us to perform quantitative image analysis. 0.2%w/v gelatin casting solutions with various IgG concentrations (0.18 µg/ml to 1.5 µg/ml) were prepared. 20 µl of each gelatin/IgG solution was cast in an area of 4.8 mm × 4 mm on a PMMA substrate. After drying, gelatin layers containing different amounts of IgG were obtained. The fluorescence images of those layers were taken and the integrated intensity of the entire layer was calibrated against known amount of embedded IgG (according to supplier information), shown in Fig. S4. In dry layers, we detect 0.51 camera units per second (CPS) per IgG molecule. With this quantitative information, the integrated intensity of an identified

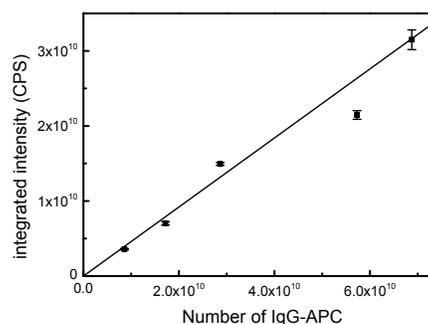cell in a fluorescence image can be used to determine the number of IgG-APC bound to that cell.

Figure S4: Calibration of the fluorescence intensity of the layer against known amounts of embedded IgG. Data points represent mean ± standard deviation (n=3).

FT-IR spectroscopy

A 0.45%w/v gelatin solution was prepared. 50 µl of the solution was cast in areas of 10 mm × 10 mm on gold substrates. Two drying conditions, ~85% RH, 4°C and ~40% RH, 45°C, were applied to obtain "cold" and "hot" gelatin layers, respectively. The infrared absorption spectra of gelatin layers were acquired using a Nicolet 6700 ATR-FT-IR spectrometer (ThermoScientific).
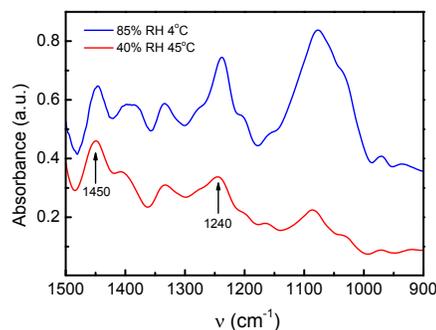

Figure S5: Representative FT-IR spectra of gelatin layers prepared under different conditions.

Differences in absorption at a characteristic band, 1240 cm⁻¹ assigned to triple helix formation[1], were observed when comparing "hot" and "cold" films. The 1240 cm⁻¹ peak height was normalized with respect to the 1450 cm⁻¹ band, associated with the protein backbone and as such not sensitive to conformational changes. It can be clearly seen in Table S1 that for samples prepared at high temperature (45°C) the 1240 cm⁻¹/1450 cm⁻¹ ratios are much lower than for samples prepared at lower temperature (4°C) corroborating our assumption that we can fine tune materials properties by changing the conditions under which layers are prepared.

Table S1: FT-IR absorption ratios of A_{1240}/A_{1450} (mean ± standard deviation, n=3) for gelatin layers dried under different conditions.

drying conditions	A_{1240}/A_{1450}
85% RH 4°C	0.58±0.03
40% RH 45°C	1.58±0.16

Reference

1. P. L. Gordon, C. Huang, R. C. Lord and I. V. Yannas, Macromolecules, 1974, 7, 954-956.