Supplementary Material

Effect of glycerol, calcium and transglutaminase post-treatment on the properties of regenerated fibers from rennet-treated casein micelles

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Transglutaminase treatment

The fibers were modified in a post treatment step, by placing them in a TGase solution at 30°C for 24 h. The TGase concentration was 1% (w/w) which corresponds to and activity of 0.8-1.35 U/g. The fibers treated with TGase were stable in NaOH, and in contrast to untreated fibers did not dissolve. The microscopy images in Figure S1 illustrate the difference between treated and untreated fibers and clearly show the stability of the treated fibers. This increased stability against NaOH hydrolysis confirms our assumption that a modification by TGase took place under the given reaction conditions.

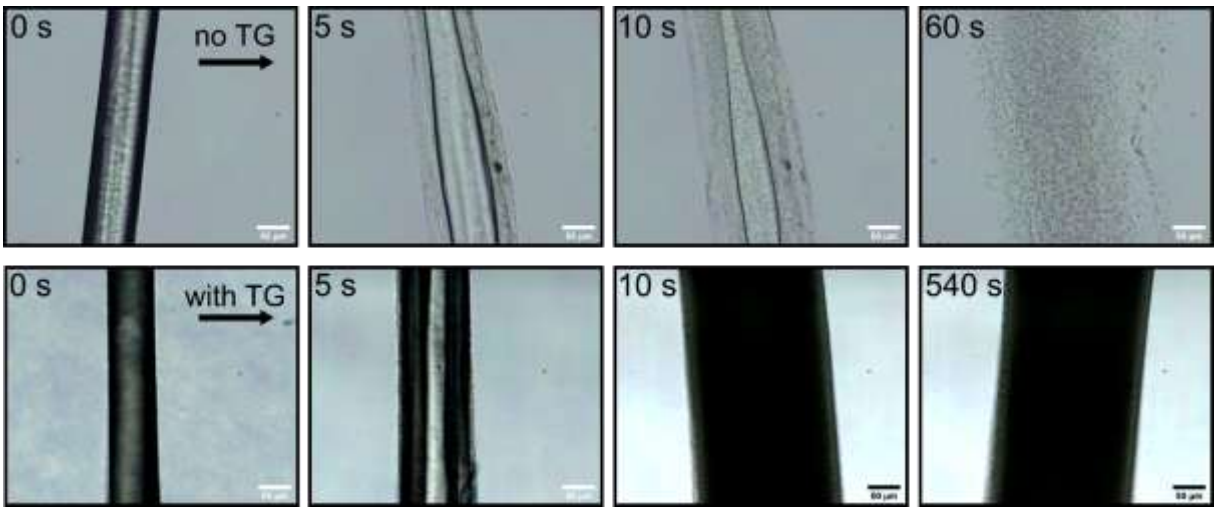

Figure S1: Microscopy images of fibers with (top) and without (bottom) TGase treatment at different times after NaOH addition. The untreated fibers completely disintegrate after about 60s whereas the treated fibers remain intact after a rapid swelling.

Figure S2 compares the swelling behavior in SMUF of fibers with- and without TGase post-treatment. The data is based on at least three repetitions and the curves significantly deviate, indicating a modification during TGase post-treatment.

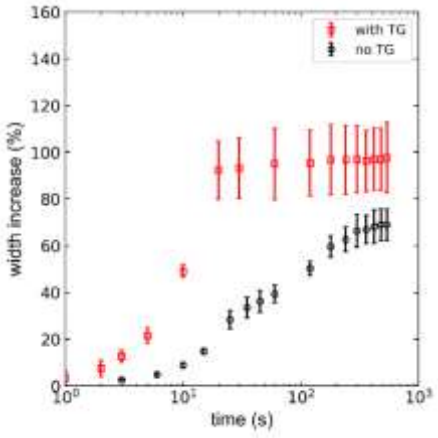

Figure S2: Swelling behavior in SMUF of fibers with and without TGase post treatment

Figure S3 compares the swelling behavior of fibers post treated 1 wt% TGase and 1 wt% deactivated TGase. The fibers treated with deactivated TGase completely dissolve in NaOH whereas the TGase treated fibers remain stable after swelling due to solvent uptake.

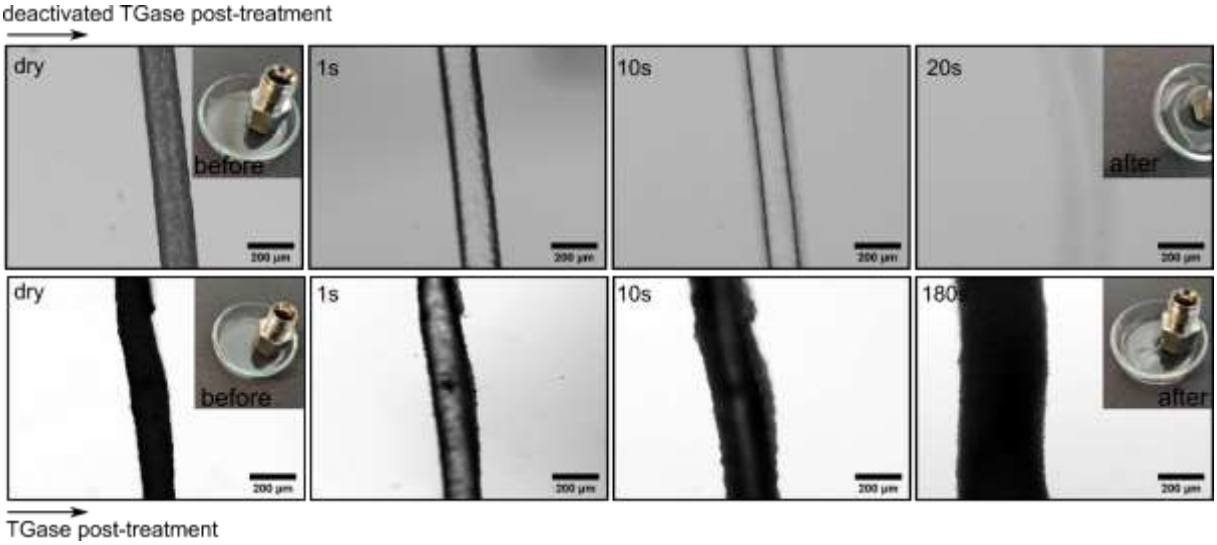

Figure S3*:* Swelling behavior of CM Fibers in 1M NaOH. Top: Fiber post treated in deactivated TGase. The fibers completely dissolve after about 20s. The inset in the first and last image shows that the fiber has completely dissolved after NaOH exposure. Bottom: Fiber post treated in TGase. The fiber exhibits a strong swelling but remains its structural integrity after NaOH exposure.

Fluorescence Micrographs

Fluorescence micrographs of fibers were taken on a TCS SP8 CLSM (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a HC PL APO CS2 93x/1.30 GLYC objective (Leica Microsystems GmbH, Wetzlar, Germany). 10% of the Casein Micelles were labelled with Atto 488-NHS (3.4 mM; Atto-tec GmbH in DMSO, dried; Merck Chemicals GmbH, Darmstadt, Germany) prior to rennet addition. Fibers spun in 50 mM $CaCl_2$ (Figure S4 b), show a more open, coarser fine structure than fibers prepared in 100 mM $CaCl_2$ (Figure S4, a).

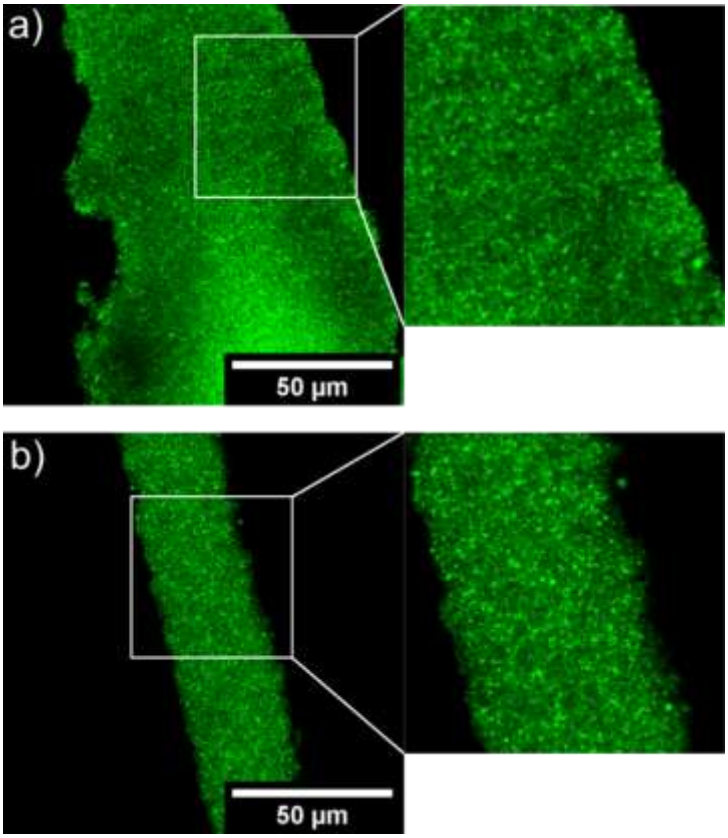

Figure S4: Confocal micrographs of fibers fabricated in a) 100mM $Cacl_2$ and b) 50mM $CaCl_2$ + 50mM $MgCl_2$, swollen in water.

Tensile Tests

The fibers show a very brittle behavior and break easily during the preparation of the tensile tests. Figure S5 shows an exemplary set of stress strain curves. The Young's modulus is obtained by a linear fit between 0.3% and 1% strain.

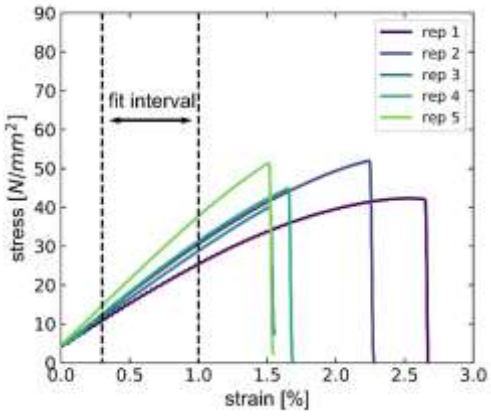

Figure S5: Exemplary stress strain curves. The Young's modulus is obtained via a linear fit between 0.3 and 1% strain (interval indicated by dashed lines).

Statistical Analysis

The complete dataset for the ANOVA analysis with means and standard deviation of Young's moduli in [GPa] is given in Table S1. The very brittle behavior of the fibers led to an unbalanced dataset with

different numers of observation for each combination of glycerol and TGase treatment. The analysis was performed using Type II Sums of squares.

Table S1: Full dataset for the ANOVA analysis with mean and standard deviation of the elastic moduli.

TG	Glyc	N	mean	stdev	min	Quart 25	Quart 75	max
yes	0%	4	2.89	0.141	2.65	2.72	2.99	2.99
yes	1%	7	1.63	0.521	0.96	0.97	2.17	2.45
yes	10%	7	1.88	0.411	1.31	1.48	2.42	2.48
no	0%	3	3.46	0.141	3.27	3.27	3.60	3.60
no	1%	6	2.71	0.999	1.32	1.61	3.66	3.81
no	10%	5	2.65	0.702	1.81	2.03	3.38	3.86

The results of the ANOVA are given in Table S2. Both, the glycerol content and the TGase post-treatment have a significant effect on the elastic modulus. However, the combination of both is not statistically significant.

Table S2: Results of the ANOVA analysis.

Variation	SS	df	F	p
Glycerol content	5.3603	2	5.9697	0.00736
TGase treatment	5.7055	1	12.7089	0.00144
Interaction	0.3275	2	0.3648	0.69784