Supporting Information

Super-Strong Hydrogel Composites Reinforced with PBO Nanofibers for Cartilage Replacement

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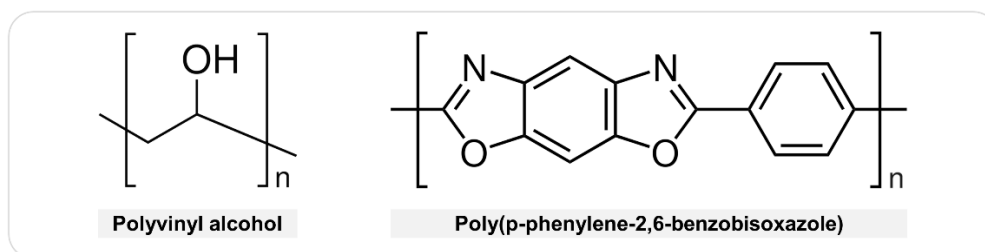

Figure S1. Chemical structure of repeating units for PVA and PBO.

A. Additional notes and comments on the preparation of PVA-PBO samples

To obtain our PVA-PBO composites, it was necessary to keep the concentration of the solutions (prepared under the conditions described in section **2.1** of the main manuscript) in the following ranges: 5–7.5% w/v for PVA; ≤1.33% w/v for PBO; and 3.1–3.67% w/v for PVA+PBO. Higher concentration of the precursor solutions (PVA or PBO) or dissolution times longer than 24 h (in the case of PVA) limited or prevented their handling at some point due to the significant increase in viscosity. Furthermore, the gelation process of the polymer mixtures was faster the higher the concentration of the respective solutions, making mixing and molding impossible when their concentration was higher than 3.67% w/v. Concentrations of the mixing solutions lower than 3.1% w/v resulted in grooves opening on the hydrogels' surfaces during the gelling period.

Another relevant point is that unlike the PVA-PBO composites, the PVA hydrogels showed some softening during the washing time. If left in water for more than three days, they tend to

gradually redissolve at RT, which is not a characteristic behavior of a fully hydrolyzed PVA[1] that is typically insoluble in water at ≈20°C. This phenomenon could be explained due to the affinity of TFA with PVA, as it can interact with it through hydrogen bonds and cause a plasticizing effect on the polymer.[2] During washing, the TFA molecules retained between the PVA chains must have been replaced by water molecules, causing the polymer chains to soften. Nevertheless, since the materials were subsequently heat-dried, this should have allowed the hydrogen bonds between the PVA chains to regenerate as the remaining solvents evaporated. In fact, no redissolution occurred after rehydration of the prepared PVA samples.
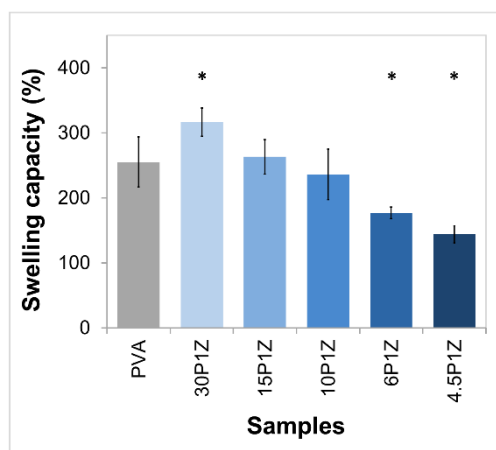

Figure S2. Equilibrium swelling in water of the materials. The error bars correspond to ± SD. Statistical significance was calculated by ANOVA and Dunnett tests. The asterisk (*) indicates the statistical difference for the comparison between samples without nanofibers (PVA - control group) and with different PVA:PBO nanofiber mass ratios ($p<0.05$).
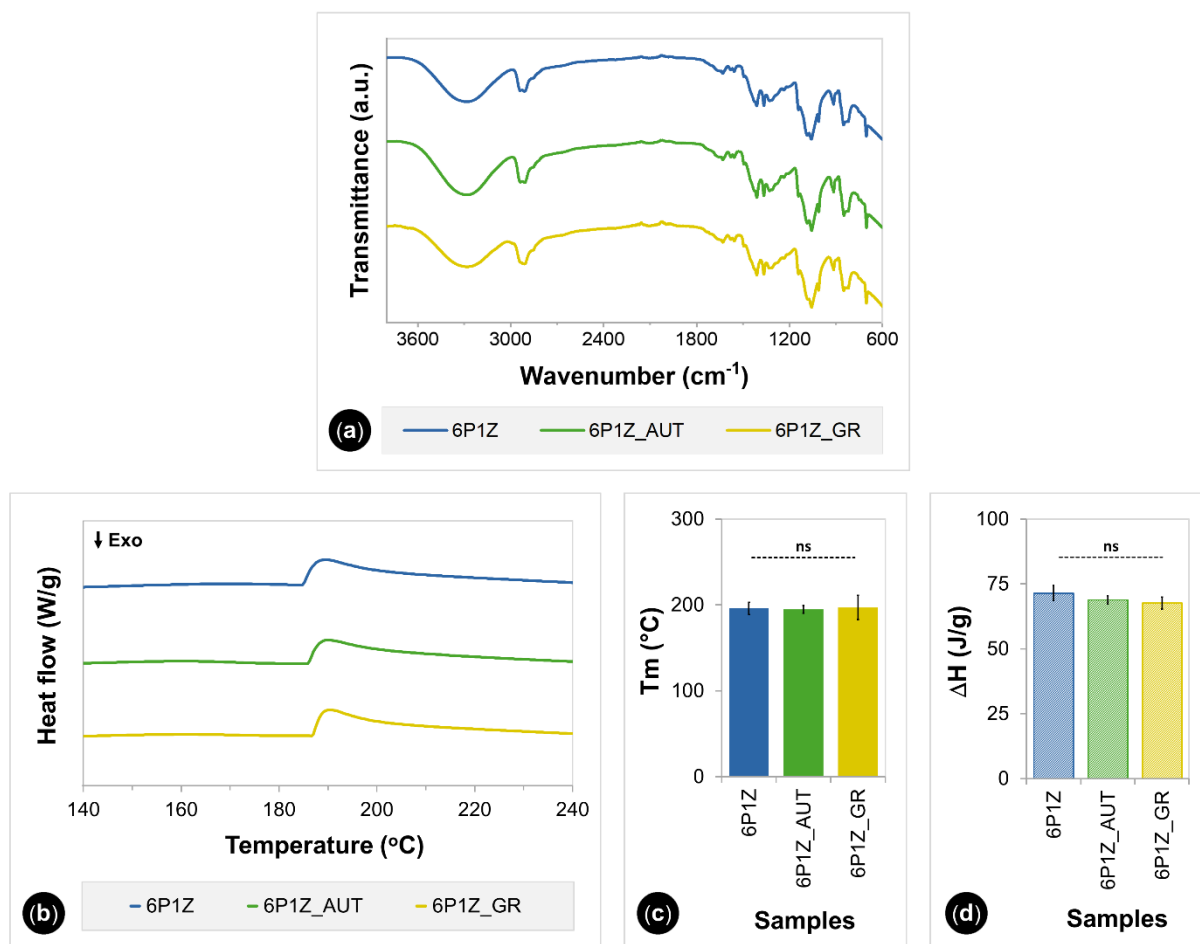
Figure S3. Chemical and thermal characterization of 6P1Z before and after sterilization by autoclaving (6P1Z_AUT) or gamma radiation (6P1Z_GR). ATR–FTIR spectra (**a**). DSC thermograms showing the melting point region (**b**) and corresponding values of the melting temperature peaks (**c**) and fusion enthalpies (**d**). The error bars correspond to ± SD. For melting temperature and fusion enthalpy data, comparisons between the non-sterilized (6P1Z - control group) and sterilized samples were performed by Welch's ANOVA and Dunnett's T3 tests. 'Ns' indicates a non-significant difference ($p \geq 0.05$).
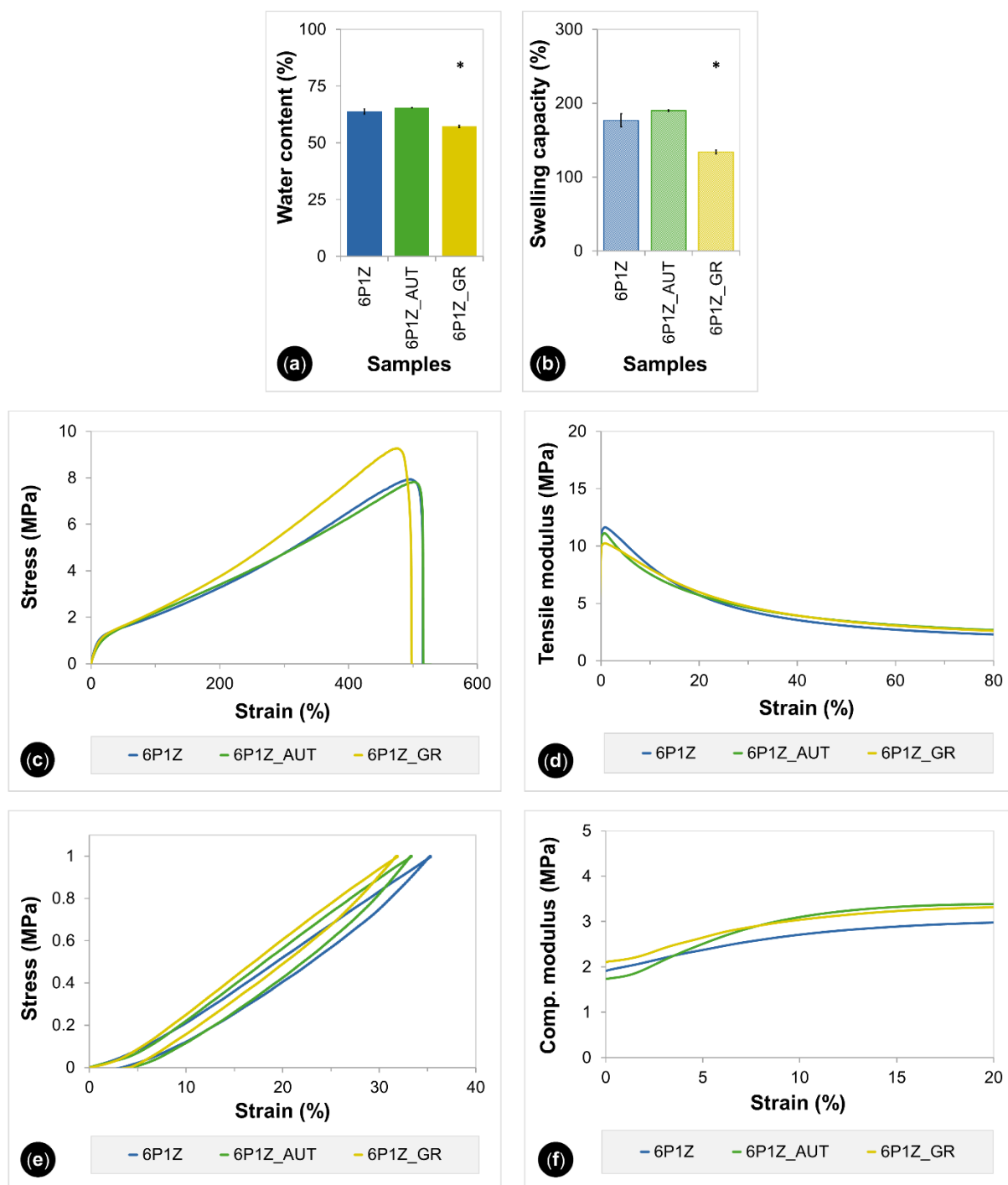
Figure S4. Physical and mechanical characterization of 6P1Z before and after sterilization by autoclaving (6P1Z_AUT) or gamma radiation (6P1Z_GR). Water content (**a**) and swelling capacity (**b**). Typical tensile stress-strain curves (**c**) and corresponding values of tensile moduli up to 80% of strain (**b**). Typical compressive stress-strain curves (**e**) and corresponding values of compression moduli up to 20% of strain (**f**). The error bars correspond to ± SD. For water content and swelling data, comparisons between the non-sterilized (6P1Z - control group) and sterilized samples were performed by Welch's ANOVA and Dunnett's T3 tests. Significant differences ($p<0.05$) between groups are indicated with an asterisk (*).

Table S1. Tensile and compression properties 6P1Z before and after sterilization by autoclaving (6P1Z_AUT) or gamma radiation (6P1Z_GR). Data are presented as means ± SD. Statistical significance was calculated by Kruskal-Wallis (UTS data) or ANOVA (all other data). All properties were not statistically different between sample groups ($p \geq 0.05$).

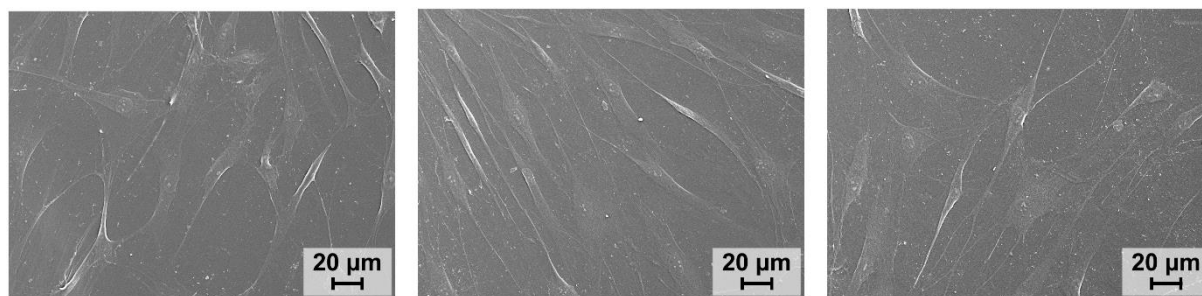| Sample	Tensile properties			Compression properties	
	Elongation at break (%)	Tensile strength (MPa)	Toughness (MJ/m^3)	Strain (%)	Dissipated energy (%)
6P1Z	518 ± 33	8 ± 1	22 ± 4	33 ± 3	20 ± 1
6P1Z_AUT	499 ± 42	8 ± 1	22 ± 4	30 ± 2	21 ± 2
6P1Z_GR	409 ± 22	9 ± 1	25 ± 3	30 ± 4	18 ± 1

Figure S5. SEM micrographs of chondrocytes on 6P1Z_AUT composite after cell culture for 7 days.
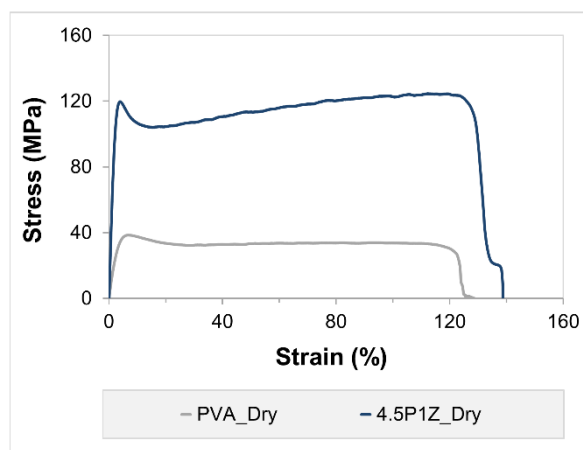
Figure S6. Typical tensile stress-strain curves of PVA and 4.5P1Z samples in the dry state.

Table S2. Tensile properties of PVA and 4.5P1Z samples in the dry state. Data are presented as means ± SD. Pairwise comparisons were performed using the independent samples t-test. Significant differences ($p<0.05$) between groups are indicated with an asterisk (*).

Sample	Tensile properties			
	Tensile modulus (MPa) at linear region	Elongation at break (%)	Tensile strength (MPa)	Toughness (MJ/m^3)
PVA_Dry	1134 ± 47	131 ± 9	39 ± 1	40 ± 2
4.5P1Z_Dry	5019 ± 354 (*)	137 ± 15	129 ± 5 (*)	147 ± 30 (*)

References

[1] C. M. Hassan, N. A. Peppas, in *Biopolym. · PVA Hydrogels, Anionic Polym. Nanocomposites* (Ed: Advances in Polymer Science), Springer, Berlin, Heidelberg, **2000**, pp. 37–65.

[2] S. Guzman-Puyol, L. Ceseracciu, J. A. Heredia-Guerrero, G. C. Anyfantis, R. Cingolani, A. Athanassiou, I. S. Bayer, *Chem. Eng. J.* **2015**, *277*, 242.