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RESEARCH ARTICLE

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Gamma Radiation Induced Contraction of Alkyne Modified Polymer Hydrogels

*Tobias G. Brevé, Huanhuan Liu, Antonia G. Denkova, and Rienk Eelkema**

Gamma radiation triggered secondary crosslinking of dextran hydrogels leads to macroscopic hydrogel contraction. The authors use stable polymer hydrogels, prepared through azide-alkyne crosslinking, containing surplus alkyne groups. γ-irradiation of these gels leads to more alkyne crosslinking, enabling controlled increase of crosslink density, which in turn leads to an increase of hydrogel stiffness and macroscopic hydrogel contraction. Gel contraction scales linearly with the applied radiation dose. The same mechanism is applied to achieve γ-radiation triggered release of the small molecule cargo, akin to wringing out a sponge. γ-irradiation of touching hydrogel objects leads to gel fusion and the formation of a self-supporting gel connection, demonstrating the reactivity of the excess alkyne groups. They envision applications in gel gluing and the construction of complex gel architectures, as well as in responsive materials for controlled release.

1. Introduction

Polymer hydrogels are formed by physically or chemically crosslinking hydrophilic polymers, with the degree of crosslinking having a large impact on the mechanical and chemical properties of the hydrogel. A wide range of methods exists to make chemical crosslinks, including photo polymerization, radical induced crosslinking, and click chemistry approaches such as copper catalyzed alkyne azide coupling and Michael additions.[1] Having control over the crosslink density enables control over the release of (bio) molecules, hydrogel stiffness, cellular signaling,[2] and eventually over the internal water volume. Crosslink density can be controlled by degradative or constructive molecular events, which depend on the desired application and can be controlled by triggers such as (UV) light, pH, enzymatic activity, or reactive oxygen species (ROS). Degradative processes such as triggered crosslinker cleavage[3] are typically employed to release (bio) molecules from the hydrogel matrix. In contrast, in constructive molecular events the crosslink density is increased, through sequential or stepwise crosslink strategies such as secondary radical-mediated crosslinking[2b] or by sequential photo-induced crosslinking.[2] Crosslink density increase is typically employed for increasing mechanical properties such as stiffness or yield stress, or to heal damage. Here, we present a γ-radiation triggered secondary crosslink strategy that enables us to have direct control over the crosslink density, after formation of the initial hydrogel, and eventually the macroscopic contraction of dextran hydrogels. With this finding we demonstrate that a molecular event, such as secondary crosslinking, can be translated into macroscopic motion (hydrogel contraction) (**Figure 1**). Most strategies for hydrogel contraction in literature rely on physical transitions. A well-known physical strategy for hydrogel contraction is the temperature triggered phase transition of poly(N-iso-propylacrylamide) (PNIPAM) based hydrogels. When such hydrogels are heated above 32 °C, a sharp decrease in material volume is observed which is caused by the polymer switching from a hydrophilic phase to a hydrophobic phase.[4] Alternatively, photoredox responsive hydrogels can undergo macroscopic contraction as a result of polymer chain folding. Blue light, via an excited ruthenium photocatalyst, triggers the folding of polyviologen chains in the hydrogel network resulting in a reduction of the hydrogel volume.[5] Mechanical entanglement can also be used for material contraction using a UV light driven molecular motor to entangle a polymer network.[6] Finally, enzymatic activity is employed to trigger a secondary crosslinker strategy leading to hydrogel contraction accompanied by an increased hydrogel stiffness.[7] Hydrogels find many different applications where they provide a protective environment to a loaded cargo. Responsive hydrogels loaded with a particular cargo enable controlled release of the cargo, through either an active or a passive release mechanism. Passive release is typically described by the standard Fickian diffusion model, where the hydrogel structure remains intact. On the contrary, active release can be controlled using external triggers such as (UV) light, temperature, pH, biological molecules, or oxidative stress and changes the integrity of the hydrogel network or the hydrogel completely disintegrates.

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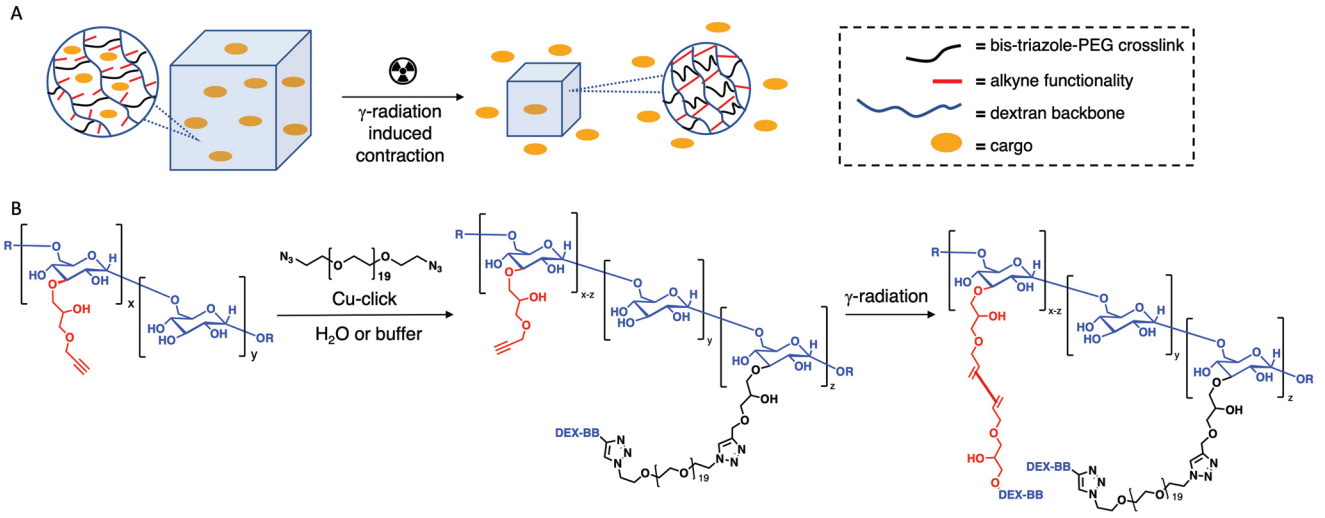

Figure 1. A) Schematic representation of cargo-loaded dextran hydrogels, which upon γ-irradiation contract and expel cargo from the hydrogel network. B) Hydrogel synthesis by crosslinking the dextran backbone (DEX-BB) and bis-azide-PEG_{19} (PEG = polyethylene glycol) using standard Cu-click conditions ($CuSO_4$, sodium ascorbate, activating ligand: tris(benzyltriazolylmethyl)amine (THTPA)). Residual alkynes are further crosslinked by γ-irradiation, resulting in contraction.

A less common trigger is ionizing radiation. A few examples exist where γ-radiation induces scissions in dendrimer structures,[8] generates reactive oxygen species which damage the bilayer of liposomes and promote cargo release[9] or the cleavage of doxorubicin from nanoparticle drug carriers.[10] γ-radiation is a powerful tool to generate radicals on unsaturated polymer chains, leading to crosslink formation, which is widely applied.[11,12] Crosslinking by gamma radiation is an efficient technique to form hydrogels as no monomers, initiators, or catalysts are used, which are potentially harmful or toxic and are thus problematic when these hydrogels find a biological application. In general, the crosslink density can be controlled by varying the radiation dose, this enables control over the degree of swelling and material properties such as stiffness.[13] When prolonged irradiation is used, the formed material continues to form crosslinks which eventually results in material contraction. This effect was observed by Angelini et al., who reported material contraction when 3% gelatine solutions were exposed to a γ-irradiation dose higher than 50 kGy.[14] In our research we demonstrate contraction of stable pre-crosslinked hydrogels, where γ-irradiation leads to immediate contraction, indicating high sensitivity.

2. Results and Discussion

Here, we present a method to contract dextran hydrogels using a covalent secondary crosslink strategy using γ-irradiation as an external stimulus. We have selected the polysaccharide dextran (500 kDa) and a polyethylene glycol (PEG) crosslinker for the preparation of pre-crosslinked hydrogels, as both polymers are biocompatible and are easily chemically modified by introducing alkyne click chemistry functionalities. Dextran is well known for applications in drug delivery and biomedical materials.[15] We started from dextran (M_W = 500 kDa) that is randomly modified with terminal alkyne side chain groups (degree of substitution = 36%, Figure S1, Supporting Information) using propargyl glycidyl ether chemistry. We then formed a hydrogel by chemically crosslinking a fraction of the alkyne moieties (theoretical maximum is 8%) via copper catalyzed azide alkyne cycloaddition with a bis-azide-PEG_{19} crosslinker (Figure 1B). This procedure afforded transparent, self-supporting water swollen hydrogels with a storage modulus (G') of 1.6×10^3 Pa and tan δ (G''/G') of 8.0×10^{-3} (**Figure 2**E). Next, we exposed centimeter-sized gel cubes to γ-irradiation from a ^{60}Co source, at doses up to 14.4 kGy. We observed that the gel cubes would shrink considerably from the start of the experiment with increasing dose (Table S1, Supporting Information), and that these contracted gels had an increased stiffness relative to their initial stiffness (Figure 2A and Figure S3, Supporting Information). Control hydrogels remained virtually unchanged during the course of the 24-hour experiment, whereas the irradiated hydrogels linearly reduced in weight (Figure 2C). The contraction rate of hydrogels swollen in phosphate buffer (PB) is lower (Figure 2B, blue data) compared to the contraction rate of hydrogels swollen in demineralized water (Figure 2B, black data). After 24 h irradiation (14.4 kGy) the weight reduction of the PB swollen gels and the demineralized water swollen gels is 39% and 74%, respectively. Figure 2F,G shows a set of photographs that illustrate the volume reduction. Figure 2G shows a transparent water swollen hydrogel at the start of the γ-irradiation experiment (left) and the contracted hydrogel after 24 h of γ-irradiation (right). During hydrogel contraction, the gels remain transparent and contract in all three dimensions equally, holding their cubic shape. A similar but less pronounced effect is observed for the PB swollen hydrogel (Figure 2F, left the hydrogel at $t = 0$ and right at $t = 24$ h γ-irradiation). We then conducted a frequency sweep experiment on the rheometer to determine the rheological properties of the hydrogel before and after γ-irradiation. The storage modulus (G') increased a factor threefold (PB swollen) or twofold (H_2O swollen) after 24 h of irradiation, indicating a more elastic material which is probably a result of additional crosslink formation (Figure 2B and Figure S3, Supporting Information). After pre-crosslinking the initial hydrogel cubes (Supporting Information Section 2.2), the hydrogels are

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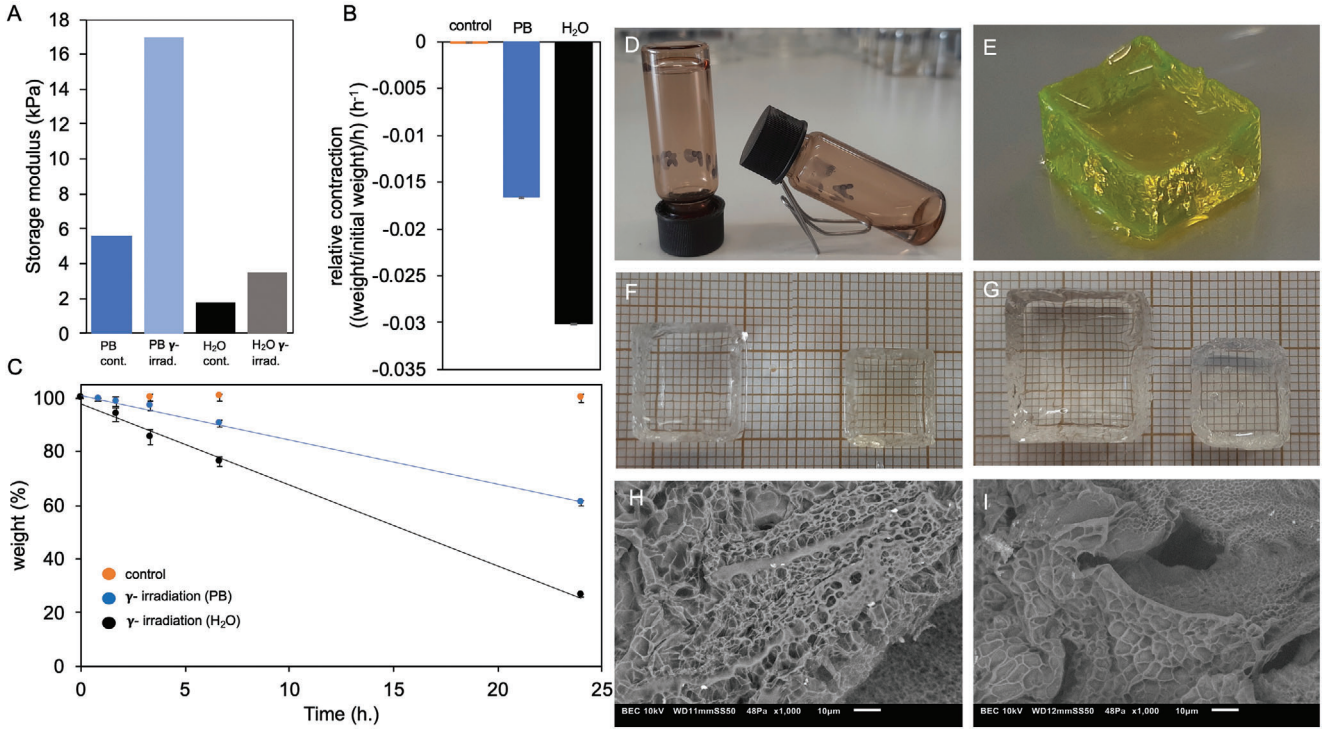

Figure 2. A) Rheology data showing an increased hydrogel stiffness after γ-irradiation. Blue data: PB swollen gels. Black data: H_2O swollen gels. B) Relative contraction rate, control: non-irradiated samples, blue: PB swollen hydrogels, black: H_2O swollen hydrogels. C) Weight change of hydrogel cubes (H_2O swollen: 1.9 cm^3 and PB swollen: 0.6 cm^3 typical size) upon γ-irradiation (^{60}Co 0.6 kGy/h). Orange data: non-irradiated samples, blue data: phosphate buffer (100 mM, pH 7.4) swollen hydrogels, black data: water swollen hydrogels. Blue and black data fitted by standard linear regression model. D) Inverted vial test. 14.4 kGy γ-irradiation resulted in gelation (in H_2O) of alkyne modified dextran (left) but did not gel unmodified dextran (500 kDa, right). E) Water swollen, fluorescein colored centimeter scale dextran hydrogel. F) PB swollen hydrogels. Photographs taken before (left) and after 24 h irradiation (right). G) H_2O swollen hydrogels. Photographs taken before (left) and after 24 h irradiation (right). H) SEM image taken before irradiation. Hydrogel was prepared and swollen in H_2O. I) SEM image taken after 24 h irradiation. Hydrogel was prepared and swollen in H_2O.

either swollen in PB or in demineralized water. The degree of hydrogel swelling is strongly affected by the ionic strength of the swelling solution and in particular if strongly kosmotropic anions (SO_4^{2-}, HPO_4^{2-}), defined by the Hofmeister series, are used. In general, as the molar concentration of kosmotropic anions increases, a salting out effect is observed for macromolecules including synthetic polymers, resulting in a lower degree of hydrogel swelling.[16] This effect becomes more pronounced when the macromolecule is (partially) nonpolar and dissolved in aqueous media. Our polysaccharide is modified with apolar alkyne side chains (degree of substitution is 36%), leading to a significant salting out effect of the dextran backbone which explains the observed difference in the degree of hydrogel swelling (1.9 cm^3 H_2O swollen compared to 0.6 cm^3 PB swollen, Figure 2F,G). Moreover, when the hydrogel matrix is less swollen, the stiffness (G′) will be higher as the crosslink concentration is higher, explaining the higher stiffness observed for the PEG_{19} pre-crosslinked hydrogels swollen in PB compared to the same hydrogels swollen in H_2O (Figure 2A). Additionally, the higher initial hydrogel stiffness observed for the PB swollen hydrogels might explain why these gels contract less compared to the H_2O swollen gels, as a stiffer hydrogel has a higher resistance to deformation.

Additionally, the hydrogels were analyzed by scanning electron microscopy (SEM) before and after γ-irradiation. SEM analysis revealed that the hydrogels have multiple morphologies which have different pore sizes (Figure S4, Supporting Information). To demonstrate the effect of γ-irradiation on the micro-scale sized pores, a similar area in a non-irradiated hydrogel and an γ-irradiated hydrogel are shown in Figure 2H,I. In the non-irradiated control hydrogel, the pore structure has a more open character compared to the γ-irradiated hydrogel. This implies that additional crosslinks are formed induced by γ-irradiation which is in agreement with the increased hydrogel stiffness (Figure 2A and Figure S3, Supporting Information). To get more insight in the mechanism of γ-irradiation induced contraction, a control experiment was conducted in which solutions of unmodified dextran (500 kDa, 10 wt% in H_2O) and alkyne modified dextran were subjected to γ-irradiation (^{60}Co source, 0.6 kGy/h) (Figure 2D). No changes could be observed for the unmodified dextran solution. In contrast, we found that the alkyne modified dextran solution gels overnight (14.4 kGy), which implies that the alkyne functionalities are crucial for hydrogel formation, and thus likely play a role in the observed contraction and increased stiffness. Crosslink formation may occur via the formation of reactive terminal alkyne radicals, which could be generated directly by γ-irradiation or indirectly via the reaction products which emerge from water radiolysis. The main products of water radiolysis are hydrated electrons, HO• (hydroxyl radical), H_2, H_2O_2, and the HO_2• (hydroperoxyl radical). The HO• is the most abundant radical which can further react and

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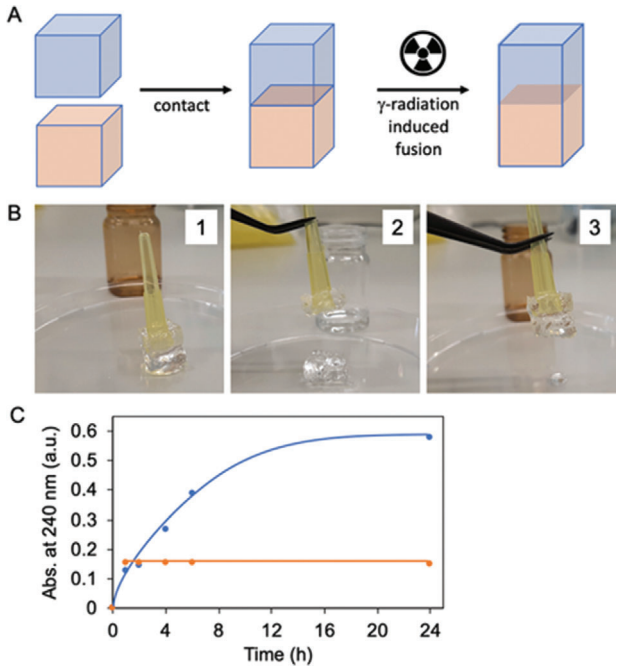

Figure 3. A) Fusion of separate dextran hydrogel cubes using γ-irradiation. B) Two gel blocks pressed together (1) do not adhere in the control setting (2), 24 h, no irradiation). When irradiated for 24 h, the top gel can lift the bottom gel, demonstrating gel fusion (3). C) Cargo expulsion upon irradiation-induced contraction. UV–Vis data show expelled 1,4-phthalic acid (monitored at 240 nm) upon prolonged γ-irradiation (0.6 kGy/h). Orange data: non-irradiated control hydrogel. Blue data: irradiated hydrogel. Lines are to guide the eye. Orange data show a constant but non-zero absorbance due to an initial single leakage of cargo solution.

create alkyne radicals.[17] Subsequently, these alkyne radicals can form crosslinks with adjacent alkyne groups resulting in the observed hydrogel contraction. Propagation can occur via an attack on the radical C1 carbon or to the cationic C2 carbon of adjacent alkyne moieties forming new C–C bonds.[18] Another possible secondary crosslink mechanism is by an attack of a radical C1 carbon on carbon C3, C4, or C5.[19] Additionally, phosphate buffer is found to act as a radical scavenger, which might explain (together with the aforementioned increased deformation resistance) the lower degree of hydrogel contraction we have observed in our γ-irradiation experiments (Figure 2B,C).[20] The reactivity of the residual alkyne groups suggested that it should be possible to glue[21] or fuse hydrogel objects using γ-irradiation (**Figure 3**A). We designed an experiment in which two hydrogel cubes where placed on top of each other inside a closed glass vial. Prior to initial gelation, a pipet tip was placed in the liquid dextran solution to provide for an easy grip handle used for lifting the hydrogels and assessing the γ-irradiation induced fusion (Figure 3B). One set of hydrogel gel cubes was then placed in a ^{60}Co source for 24 h and one set was kept aside as a control experiment. We found that the hydrogel cubes in the control experiment did not fuse together. When lifting the top hydrogel, the bottom hydrogel immediately detached indicating that capillary forces do not play any significant role (Figure 3B-i). In contrast, the γ-irradiated set of hydrogels had fused together and could be lifted with the top hydrogel cube holding the weight of the bottom hydrogel cube (Figure 3B-iii). Based on the weight of the gel cubes (0.5 g per cube) and the size of the interface (1.0 cm^2), the adhesive strength of the newly formed connection is estimated to be at least 50 Pa (see Supporting Information for calculation). The adhesive strength may be substantially higher as this calculation assumes a perfectly connected interface which is not realistic. Finally, we were curious if we could release a loaded cargo from the hydrogel matrix, as a result of γ-irradiation triggered hydrogel contraction (Figure 3C). In this experiment, we used hydrogels loaded with model compound 1,4-phthalic acid. The hydrogel cubes were isolated from the surrounding water volume by placing them on a glass plateau inside a closed glass vial (Figure S5, Supporting Information). This experiment setup allowed us to limit passive diffusion of 1,4-phthalic acid from the hydrogel matrix and only observe the "squeezing" effect by contraction. We found that the UV/Vis absorbance (240 nm) of the water volume of the γ-irradiated hydrogel increases over time, indicating the release of 1,4-phthalic acid, while the absorbance of the water volume of the control gel stayed stable over time.

3. Conclusion

In conclusion, we here demonstrate a versatile γ-irradiation triggered hydrogel crosslinking strategy that enables control over hydrogel stiffness, contraction, release, and fusion. We found a linear relationship between the γ-irradiation dose and the degree of hydrogel contraction, with a more pronounced effect in water than in phosphate buffer. The stiffness of the hydrogels increased twofold for H_2O swollen hydrogels and threefold for PB swollen hydrogels after γ-irradiation, which is the result of the increased crosslink density. γ-irradiation triggered crosslinking enables fusion of hydrogel objects. In addition, we show that γ-irradiation triggered hydrogel contraction can be used to squeeze out a cargo. All together our finding provides for a γ-irradiation sensitive material having potential in material science and triggered release applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data openly available in a public repository DOI: 10.4121/16904590.v1

Keywords

controlled release, gamma radiation, hydrogel contraction, hydrogel fusion

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