

# Self-healing hydrogels formed by reversible interactions between calcium ions and bisphosphonate-functionalized polymers

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**Statement of Purpose:** Hydrogels are typically covalently cross-linked to avoid dissolution of the hydrophilic polymer chains. In addition to conventional cross-linking strategies employing toxic chemicals, the irreversibility of these covalent bonds renders such materials incapable of self-healing since these bonds cannot be restored after rupture. Therefore, there has been increasing interest in finding alternative hydrogel crosslinking mechanisms that are more benign, and allow for recovery of properties after rupture.

Bisphosphonates are a group of well-established drugs widely used for the treatment of metabolic bone diseases, including hypercalcemia and osteoporosis. These drugs exhibit a strong affinity to calcium cations as present in bone mineral hydroxyapatite [1]. We hypothesized that covalent attachment of bisphosphonate groups to synthetic, hydrophilic polymers allows the formation of bisphosphonate-grafted hydrogels by means of cross-linking with multivalent cations. Therefore, the aim of the current study was to investigate if poly(ethylene glycol)-based hydrogels can be cross-linked by the formation of reversible bonds between bisphosphonate-functionalized multi-arm poly(ethylene glycol) (PEG) and multivalent cations.

**Methods:** The end groups of 4- or 8-arm, star-shaped PEG macromers were functionalized to yield N-hydroxysuccinimide esters (PEG-NHS) as previously described [2]. Amino-bisphosphonate alendronic acid (ALN) was covalently linked to the NHS-functionalized PEG by reacting the free amines of ALN with the active esters of PEG-NHS yielding alendronate-functionalized PEG (PEG-ALN). The degree of modification was followed by proton nuclear magnetic resonance (<sup>1</sup>H-NMR) and the final bisphosphonate concentration was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES).

Ionically cross-linked hydrogels were formed immediately after mixing polymer and CaCl<sub>2</sub> solutions. The viscoelastic properties of these gels were studied using oscillatory rheology as a function of Ca<sup>+2</sup> concentration (50 to 500mM) and polymer concentration (10 to 20 w/v%), molecular weight (10 to 40 kDa) and number of arms (4 or 8) of the polymer. The efficacy of other divalent cations (i.e. Co<sup>+2</sup>, Co<sup>+2</sup>, Mg<sup>+2</sup>, Cu<sup>+2</sup>, Mn<sup>+2</sup>, Ni<sup>+2</sup>, Sr<sup>+2</sup> and Zn<sup>+2</sup>) to cross-link PEG-ALN was also evaluated using rheology. The relaxation time of the gels (defined as the inverse of the frequency at G' and G'' cross-over point) was measured using frequency sweeps (100 to 0.1 rad/s).

**Results:** The functionalization of 8-arm (10kDa, 20kDa and 40kDa) and 4-arm (10kDa) PEGs with alendronic acid was efficient as evidenced by a degree of substitution

of about 85% as shown by <sup>1</sup>H-NMR and ICP-OES. Ca<sup>+2</sup>-crosslinked hydrogels were moldable and self-healing as indicated by rapid rheological recovery of storage modulus following rupture at high strain (Figure 1). After cutting of hydrogel samples, recovery of gel cohesion was instantaneous and autonomous upon fusion of separated parts (Figure 1).

Storage moduli increased with increasing calcium and polymer concentrations up to values of 6.5 kPa at a polymer content of 15 w/v% and a calcium concentration of 200 mM. Calcium concentrations higher than 200 mM, however, resulted into decreased storage moduli similar to other ionically crosslinked hydrogels [3]. The hydrogels formed by interaction with calcium cations displayed the highest storage moduli when compared to all other multivalent cations, confirming that calcium formed the strongest interaction with bisphosphonate. Accordingly, gel relaxation times followed the tendency: Ca<sup>+2</sup> > Zn<sup>+2</sup> > Co<sup>+2</sup> > Ni<sup>+2</sup> > Sr<sup>+2</sup> > Mn<sup>+2</sup>.

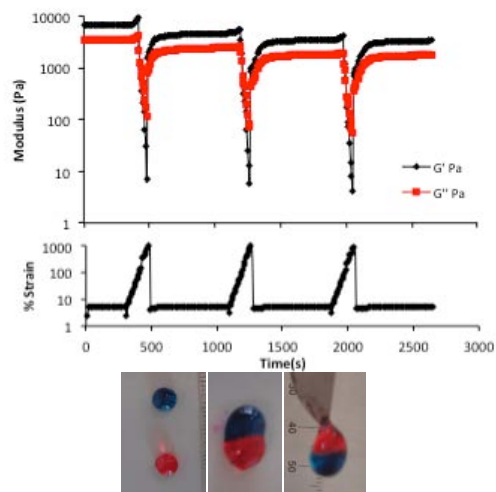


Figure 1. Self-healing of PEG-ALN hydrogels containing 15 w/v% polymer and 100mM Ca<sup>+2</sup>. The hydrogel was subjected to a destructive strain of 1000% followed by monitoring the recovery of the storage modulus at 5%. The photos show self-healing of artificially colored gel fragments.

**Conclusions:** Self-healing gels were formed by reversible interactions between bisphosphonates covalently attached to multi-armed PEG with soluble calcium cations. Gel elasticity increased with polymer content and calcium concentration, while calcium-mediated crosslinking was shown to be superior over cross-linking mediated by other divalent cations.

## References:

- [1] Wilkinson J.M., Bone, 2011. 49(1):95-102.
- [2] Strehin I., Biomater.Sci., 2013;1:603-613
- [3] Topuz F., Soft Matter, 2012;8:4877-4881