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Design of moldable hydrogels for biomedical applications using dynamic covalent boronic esters

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ABSTRACT

Traditional polymeric materials based on thermosets or thermoplastics are applied broadly as biomedical materials. While attractive for a range of applications, thermosets and thermoplastics can be limited by their relatively static materials properties. Recent efforts in materials design has focused on engineering responsive and adaptive networks based on dynamic covalent chemistries. Installing reversible chemistries within the network backbone enables breaking and reforming of bonds in the network and associated rearrangement of the material on experimental timescales. The complexation between boronic acids and diols to form reversible boronic esters has emerged as a safe and synthetically tractable dynamic covalent cross-linking motif for the design of stimuli-responsive biomedical materials. Here, we present an instructive review on the design of dynamic covalent networks and gels using boronic ester cross-links. We provide a detailed discussion of boronic ester chemistry with guidelines for tuning the binding based on synthetic modification. We explain how network topology and connectivity influence the macroscale properties of the assembled networks. In addition, we discuss how these design principles have been used in foundational and emerging biomedical applications of boronic ester–based hydrogels. The use of boronic esters as dynamic covalent cross-links will continue to produce materials with emergent dynamic properties, and the design principles presented here will aid in the fabrication of next-generation boronic ester-based biomaterials.

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1. Introduction

Dynamic covalent chemistries offer an attractive route for soft materials fabrication, where the strength of covalent bonds is combined with the reversibility of non-covalent interactions [1]. Polymer networks are formed through physical or chemical cross-linking of functional monomeric or polymeric precursors. Physically cross-linked networks are held together with non-covalent, reversible interactions, such as hydrogen bonding, hydrophobic interactions, or metal-ligand coordination [2]. These often result in shear-thinning (viscous flow upon increased shear) and self-healing (reformation of gel properties after shear cessation) networks, as the bonds can break and reform in response to external stimuli, including force. However, the structures formed are often unstable to small environmental perturbations and lack robustness. Chemically cross-linked networks, on the other hand, are held together by permanent covalent bonds [3]. These give rise to elastic gels with generally higher mechanical properties as compared with physical networks, but the irreversibility of chemical cross-links limits their use for applications that require shear-thinning and self-healing, such as 3D printing or minimally invasive drug delivery.

Recent research in the design and engineering of polymer networks has introduced a new class of soft matter based on dynamic covalent chemistry, which combines the benefits of both physically and chemically cross-linked materials [4]. In this approach, reversible covalent bonds are installed in the network backbone that can break and reform on experimental timescales. Thus, these dynamic covalent networks can rearrange because of the exchange of bonds, enabling stress relaxation and material flow. Often, the binding affinity of the dynamic covalent chemistry is sensitive to changes in environmental conditions, allowing for stimuli-responsive materials properties. Chemistries that have been used in dynamic covalent network formation include transesterification [5], Diels-Alder (DA) cycloaddition [6], and boronic ester complexation [7]. These have enabled the fabrication of processable and recyclable vitrimers, temperature-induced self-healing...
networks, and injectable hydrogels, respectively. In each case, the ability to break and reform bonds at the molecular scale led to emergent dynamics at the macroscale.

In this review, we present an overview of how boronic ester bonds can be used to engineer dynamic covalent networks and gels. After introducing dynamic covalent chemistry and boronic ester bonds, we provide a thorough discussion of how chemistry influences the binding kinetics and thermodynamics for boronic ester formation and breakage. We then discuss how network formation and topology influence the macroscopic properties of dynamic covalent networks. In addition, we survey foundational and emerging biomedical applications that leverage these chemical and physical insights for materials fabrication using boronic ester cross-links. Finally, we conclude with an outlook on the future of this field.

1.1. Dynamic covalent chemistry

In dynamic covalent chemistries, the competing chemical reaction pathways leading to different products are under thermodynamic control. The structures produced are thus not only reversible but also strong and tough, since they are held together by covalent bonds. In such systems, the relative stabilities $\Delta G^\circ$ of the products determine their final distribution; rather than the relative energies $\Delta E$ required to form them, as in kinetically controlled systems [8]. As a result, slight modifications to the starting materials (constitutional, steric, or electronic) or changes to the external environment (such as pH, temperature, or concentration) can significantly alter the thermodynamic equilibrium [8]. Dynamic covalent systems thus adapt to changing conditions because their molecular components can easily assemble and disassemble into different product distributions in response to shifts in the chemical equilibrium.

Dynamic moieties can therefore be incorporated into polymeric networks, allowing for rearrangement of bond connectivity, as shown in Fig. 1. By continuously breaking and reforming bonds, dynamic covalent networks adapt to physical or chemical cues (such as mechanical loading, pH, or temperature), resulting in highly processable and self-healing materials with frequency-dependent mechanical properties. For example, disulfide cross-links (Fig. 1a) were established early on to be reversible [9,10]. Later, the formation and cleavage of disulfide bonds in response to light and pH was exploited to fabricate reversible networks [11,12]. Another common strategy to produce dynamic networks involves the DA cycloaddition reaction. Chen et al. synthesized a highly cross-linked polymer network via the DA reaction of furan and maleimide moieties [6]. Network rearrangement was achieved after heating, which favors the retro-DA reaction. In a different study, Skene and Lehner leveraged acylhydrazone functionalities in dynamic polyamides to reversibly exchange repeating monomers [13]. In each of these reactions, the sequence of bond-breaking and formation involves a transition of decreased network connectivity; a bond must be broken before another one is formed. Different mechanical properties can also be achieved when the bond-breaking sequence occurs without a loss of connectivity, as in reversible exchange reactions [4]. Prominently, Montarnal et al. formed irreversible epoxy networks that can rearrange their topology by undergoing a transesterification reaction (Fig. 1c), where the shuffling of the bonds occurs without depolymerization [5].

Critical, the kinetics associated with dynamic covalent bond formation are usually slow because the lifetime of the bonds must be long enough to produce stable and robust structures. This often requires the use of a catalyst, the application of heat, or extreme changes in pH to equilibrate the thermodynamically stable product on experimental timescales [8]. As a result, a current focus in the field of dynamic covalent networks is to find materials which are suitable for biomedical applications.

1.2. Boronic esters as dynamic covalent cross-links

Boronic ester cross-linked networks have emerged as a promising class of dynamic materials. The reversible condensation reaction between boronic acids and cis-1,2- or cis-1,3-diols to form a cyclic ester (Fig. 1b) occurs in aqueous solutions under mild conditions, at ambient temperature, and requires no catalyst [14]. This enables boronic ester formation under physiological conditions. Furthermore, the reactivity of the boronic acid groups can be tuned using simple neighboring group effects, resulting in materials with highly tuneable mechanical properties that range from mechanically static to extremely malleable [15]. Building from initial studies on the use of boronic acids in medicine, boronic esters have become a common cross-linking motif in the design of biomaterials. The behavior of these materials depends directly on the underlying binding chemistry of the selected boronic ester and rational design therefore relies on a robust understanding of how to tailor the kinetics and thermodynamics of boronic ester bond formation and breaking.

1.3. Biomedical applications of boronic acids

Boronic acids already enjoy widespread use in medicine owing to their low toxicity [16] and biofunctionality [17]. One of the earliest and best-known biomedical uses of boronic acids was as enzyme inhibitors. In 2003, the US Food and Drug Administration (FDA) approved bortezomib as the first proteasome inhibitor for human use [18]. This anticancer drug consists of a dipeptide containing a boronic acid that competitively binds to the hydroxyl groups found in the active site of serine proteases [19]. Upon binding, a reversible tetrahedral intermediate is formed which mimics the normal transition state and inhibits enzymatic activity [20].

Another important biomedical use for boronic acids has been as synthetic chemical sensors for biomolecules. In 1959, Lorand and Edwards calculated the binding affinities of various polyols to the benzeneborate ion by measuring the associated drop in pH upon combining them in aqueous solutions [21]. They showed that the reversible interaction between a boronic acid and a diol shifts when external conditions such as pH and temperature are changed, as it is under thermodynamic control. Conversely, the addition of free diols to an aqueous solution containing boronic acids would also shift the equilibrium, causing an associated change in measurable characteristics such as pH or solubility. This discovery forms the basis for the real-time molecular sensing of many biologically important diol-containing molecules. Research has focused mainly on the detection of saccharides, although sensing of other molecules such as ATP [22] or even heavy metal ions [23] has been demonstrated. A typical device design is to immobilize boronic acid moieties on a substrate. The selective binding of sugars on the device is accompanied by a shift in thermodynamic equilibrium, which results in changes in the measurable properties of the system [14]. For example, optical sensors rely on the change in UV–Vis absorption when certain boronic acids are bound to saccharides [24] while fluorescence sensors measure the intensity difference of bound and unbound fluorophore-functionalized boronic acids [25].

Several recent reviews have highlighted the extensive progress in the field of molecular sensing by boronic acids of saccharides [26,27] and of glucose in particular [28].
The broad utility of boronic acids is a direct consequence of their dynamic functionality. Interestingly, the same properties of boronic acids that make them attractive for biomedical applications have also been used to form dynamic networks cross-linked with boronic esters. The unique properties of these materials are related directly to the dynamic covalent bonding between boronic acids and diols, which will now be discussed.

2. Chemistry of boronic esters

2.1. Boronic acid chemistry

Boronic acids are weak organic Lewis acids. The ionic equilibrium in aqueous solution between the neutral and anionic forms of boronic acids is shown in Fig. 2. Under acidic conditions, the neutral form is favored, with a vacant p-orbital [29]. The B-atom is sp²-hybridized and adopts a trigonal planar configuration with an O-B-O bond angle of 120°. Under more basic conditions, the electron-deficient B-atom is attacked by OH⁻ ions, forming a hydroxyboronate anion. Upon OH⁻ ion complexation, the B-atom becomes sp³-hybridized and adopts a tetrahedral configuration with a bond angle of –109.5° [21,29]. These two species have very different electronic properties; the boronic acid is electron-accepting, while the boronate anion is electron-donating [30]. Thus, any chemical reaction involving a boronic acid is highly

\[
K_{eq} = \frac{k_c}{k_o} = \frac{[\text{boronic acid}]}{[\text{boronate anion}]}\]

Fig. 1. Dynamic covalent network formation. Moldable polymer networks and gels can be formed by cross-linking macromolecular precursors (for example with multi-arm star polymers as shown here) using dynamic covalent chemistries. The reversible cross-links can exist in the bound state (orange) or the unbound state (red and blue), and the exchange between bound and unbound is under thermodynamic control, as shown by \(K_{eq}\). Constant breaking and formation of the bonds enables network strand rearrangement and stress relaxation as well as the reversible transition between the gel and liquid state (as shown here). Dynamic covalent chemistries used for moldable network formation can be categorized as (a) addition reactions exemplified by disulfide formation, (b) condensation reactions including boronic ester formation, which is the focus of this review, and (c) exchange reactions such as transesterification.

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\[
R_1\text{B}\text{OH} + \text{H}_2\text{O} \rightleftharpoons K_{eq} \rightleftharpoons R_1\text{B(OH)}_2 + \text{H}^+ \]

Fig. 2. Ionic equilibrium of boronic acids. In aqueous solution, boronic acids exist in ionic equilibrium between their neutral and anionic forms, which possess different electronic and structural properties. The neutral form is electron-accepting with a trigonal planar configuration, whereas the anion is electron-donating with a tetrahedral configuration. Boronic ester formation occurs through complexation of the boronic acid with cis-diols, and the rate depends on the equilibrium between the neutral and anionic forms.
dependent on the equilibrium between its neutral and anionic forms.

2.2. Chemistry of boronic ester complexation

Boronic acids interact with polyols in aqueous solution to form reversible and cyclic esters [31]. Fig. 3 summarizes the reaction landscape of boronic ester formation, highlighting the possible pathways for the reaction of boronic acids with cis-diols moieties. As shown in Fig. 3, both cyclic boronic and boronate esters are formed with their B-atoms, sp²-and sp³-hybridized, respectively. The boronic and boronate esters exist in ionic equilibrium. Initially, it was believed that the boronate anion was much more reactive than the neutral acid because ester formation is favored at high pH, where the concentration of the anions are high. This was supported by kinetic studies based on the pH-depression method [21], which suggested that the boronate anion was 10⁴ times faster than the neutral boronic acid in forming esters with diols [32]. More recently, however, Springsteen and Wang revealed discrepancies with these earlier measurements when investigating boronic ester stability with the fluorescent reporter Alizarin Red S. (ARS) [33]. In fact, the precise kinetics of boronic acid-diol complexation are much more complicated than originally thought. Other factors have to be considered, such as the buffer system. For instance, a medium dependence on the binding affinities between boronic acids and diols was observed because of the formation of binary and ternary complexes with common buffer components [34]. Furthermore, some kinetic paths are indistinguishable from each other due to the effects of ‘proton ambiguity’ [35]. It is now proposed that the preferred kinetic pathway for esterification is through the addition of diols to the neutral boronic acid, rather than through substitution of the hydroxyl ion in the anionic species [30]. This would imply that the reaction rates of esterification are inversely proportional to pH. Evidence of this can be found in materials cross-linked with boronic esters, as longer gel times are observed with increasing pH of the precursor solutions [36].

To account for the multiple ionization states of the acid, ester, and diols in aqueous solution, Van Duin et al. proposed the ‘charge rule’ to determine the pH-dependent reactivity of the ester. The authors hypothesized that the maximum amount of esters are found at the pH at which the ‘sum of the charges of the free esterifying species is equal to the charge of the ester’ [37]. Since the pkₐ of a molecule defines the pH at which 50% of the neutral groups are converted to their anionic forms, this rule has been interpreted to mean that the optimal pH for ester formation is somewhere in between the pkₐ values of the boronic acid and the diol. A simple approximation to this value is to use the mean pkₐ of the boronic acid and diol, as shown in Eq. (1):

\[
pH_{optimal} = \frac{pk_{a\text{-acid}} + pk_{a\text{-diol}}}{2}
\]

The validity of this relationship has been confirmed experimentally by several studies that examined the effects of substituents on the affinity of boronic acids to diols [30,38]. Recently, a more complete general equation for this relationship was independently derived by Martínez-Aguirre et al. from equilibrium constant measurements and mass balances [39]. Given that diols are almost always less acidic than boronic acids, the charge rule implies that to find an optimal acid-diol pair for physiological use, the pkₐ of the boronic acid must be equal to or less than 7.4. To this end, boronic acids have been modified chemically to tune their pkₐ, allowing their tailored use for specific conditions and different applications.

2.3. Ionization constants (pkₐ) of boronic acids

To broaden the utility of boronic acids for ester formation in balanced pH solutions (pH ~7) and for biomedical applications, new synthetic variants of boronic acid have been designed with tailored pkₐ. Table 1 highlights several boronic acid derivatives and their associated pkₐ values as published in the literature. In general, a few design guidelines have emerged. Electron-withdrawing groups

Fig. 3. Reaction landscape of boronic ester formation. Both neutral and anionic forms of the boronic acid exist in thermodynamic equilibrium with cis-diols in aqueous solution, forming boronic and boronate esters via condensation reactions. Kₚ₉ and Kₚ₇₈ are the equilibrium reaction constants for the pathways between the trigonal neutral and the tetrahedral anionic forms, respectively. Kₐ and Kₐ’ are the ionization constants of the boronic acid and boronic ester, respectively.
lower the pKₐ of the boronic acid, whereas electron-donating groups increase its pKₐ. This is because the more acidic boronic acids contain the more electrophilic boron atoms, which improve the formation and stabilization of the boronate anion[29]. Indeed, alkylboronic acids are generally less acidic than arylboronic acids. For example, the pKₐ of methylboronic acid (10.4) is much higher than that of phenylboronic acid (PBA) (8.8). From Eq. (1), however, PBA is not ideal for physiological use as its pKₐ is greater than 7.4. To improve this, strong electron withdrawing groups have been introduced into the phenyl ring of PBAs in an attempt to lower their pKₐ[40]. Table 2 shows how the addition of substituent groups with different electronic properties significantly alters the acidity of PBA derivatives. Electron-rich derivatives of PBA such as 2-methyl and 2-methoxy PBA have higher pKₐ values (9.7 and 9.0, respectively) than electron-poor derivatives such as 3-methoxycarbonyl-5-nitro and 2-fluoro-5-nitro PBA (6.9 and 6.0, respectively). Plotting the pKₐ of substituted PBAs against the Hammet ρ-values of the substituents yields a straight line with a slope of 2.1[38], indicating the formation of an anionic product as shown in Fig. 2. However, some exceptions to this trend exist. For example, the pKₐ of 2-methyl PBA is higher (9.7) than that of 4-methyl PBA (9.3), which is explained by the additional steric hindrance of the 2-methyl group during complexation to the boronate anion[29].

Recently attention has focused on 2-hydroxymethyl PBA, shown in Table 1. In water, it spontaneously converts to a cyclic benzoboroxole (also known as a benzoxaborole) via intramolecular B-O coordination. This accounts for its relatively low pKₐ (7.2), which is largely because of a decrease in ring strain associated with rearrangement from the neutral sp³–hybridized form to the anionic sp³–hybridized form. Other key advantages of benzoboroxoles are their high water-solubility and their ability to bind to many different types of diols [41].

‘Wulff-type’ boronic acids, such as 2-aminomethyl PBA (pKₐ = 5.2) shown in Table 1, are commonly used for the sensing of saccharides [41]. In aqueous solution, a dative bond is formed between the N and B atoms in these types of acids, which enhances binding at neutral pH [42]. The exact reasons behind this lower pKₐ are still debated, with some citing a shift to sp³–hybridized boron after the B-N adduct is formed while others believe that there is ionic stabilization of the boronate center by a cationic ammonium center [43]. Finally, zwitterionic and heteroaromatic compounds, such as 3-pyridylboronic acid, shown in Table 1, are known to have pKₐ values as low as 4.0[44]. Therefore, with the appropriate chemical modification, the pKₐ of various boronic acids can be varied from ~4.0 to 10. As a result, the equilibrium of esterification can be shifted across a range of pH, dictated by the pKₐ of the boronic acid derivative. This enables complexation in acidic and basic environments, including under physiological conditions. It is worth keeping in mind that the acidity of the diol also plays a role in determining the optimal pH, especially in the rare case when the diol is more acidic than the boronic acid. A notable example occurs during the binding between ARS (pKₐ = 6.9) and PBA (pKₐ = 8.8), where the optimal pH was found to be ~7.0[33].

### 2.4. Binding affinities of diols to boronic acids

Another critical factor to consider for the stability of boronic ester bonds is the binding affinity of the diol to the boronic acid, which is a measure of the strength of the interaction between the

### Table 1

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Structure</th>
<th>pKₐ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boric acid</td>
<td></td>
<td>9.2</td>
<td>a</td>
</tr>
<tr>
<td>Methylboronic acid</td>
<td></td>
<td>10.4</td>
<td>b</td>
</tr>
<tr>
<td>Phenylboronic acid</td>
<td></td>
<td>8.8</td>
<td>c</td>
</tr>
<tr>
<td>2-Hydroxymethylphenyl boronic acid</td>
<td></td>
<td>7.2</td>
<td>d</td>
</tr>
<tr>
<td>2-Dimethylaminomethyl phenylboronic acid</td>
<td></td>
<td>5.2</td>
<td>e</td>
</tr>
<tr>
<td>3-Pyridylboronic acid</td>
<td></td>
<td>4.0</td>
<td>f</td>
</tr>
</tbody>
</table>

1 The references for the pKₐ values taken from literature are (a)[111], (b)[32], (c)[38], (d)[41], (e)[42], and (f)[44].
diol and the boronic acid. This can be captured by \( K_{eq} \), the equilibrium dissociation constant for the esterification reaction, which determines the composition of the system at thermodynamic equilibrium, irrespective of the starting conditions. Fig. 1 shows how \( K_{eq} \) is related to the quotient of the concentrations of the species involved in dynamic covalent network formation, as well as to the forward and backwards rate constants \( k_f \) and \( k_b \) of the chemical reaction [8]. In 1959, Lorand and Edwards carried out the first systematic examination of the binding affinities between boronic acids and diols. Their reported formation constants between selected polyols and PBA are listed in Table 3, under Method 1. The pH depression method used, however, cannot fully capture the complexity of boronic acid-diol binding. Their experimental design was based on the formation constant being directly proportional to the drop in pH associated upon the formation of a boronate ester. This method assumes that only the tetrahedral boronate anions are reacting with the diols; however, it is known that the neutral trigonal species also play a role. Therefore, recent studies have proposed that the binding constants measured by Lorand and Edwards are values for \( K_{tet} \) rather than the overall \( K_{eq} \).

Table 2
Ionization constants for selected phenylboronic acid derivatives.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Structure</th>
<th>( pK_a )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methylphenyl boronic acid</td>
<td><img src="image1" alt="Structure" /></td>
<td>9.7</td>
<td>a</td>
</tr>
<tr>
<td>4-Methylphenyl boronic acid</td>
<td><img src="image2" alt="Structure" /></td>
<td>9.3</td>
<td>a</td>
</tr>
<tr>
<td>2-Methoxyphenyl boronic acid</td>
<td><img src="image3" alt="Structure" /></td>
<td>9.0</td>
<td>b</td>
</tr>
<tr>
<td>4-Carboxyphenyl boronic acid</td>
<td><img src="image4" alt="Structure" /></td>
<td>8.4</td>
<td>c</td>
</tr>
<tr>
<td>2-Fluorophenyl boronic acid</td>
<td><img src="image5" alt="Structure" /></td>
<td>7.9</td>
<td>d</td>
</tr>
<tr>
<td>3-Nitrophenyl boronic acid</td>
<td><img src="image6" alt="Structure" /></td>
<td>7.0</td>
<td>e</td>
</tr>
<tr>
<td>3-Methoxycarbonyl-5-nitro phenylboronic acid</td>
<td><img src="image7" alt="Structure" /></td>
<td>6.9</td>
<td>f</td>
</tr>
<tr>
<td>2-Fluoro-5-nitrophenyl boronic acid</td>
<td><img src="image8" alt="Structure" /></td>
<td>6.0</td>
<td>b</td>
</tr>
</tbody>
</table>

1. The references for the \( pK_a \) values taken from literature are (a) [29], (b) [38], (c) [113], (d) [114], (e) [115], and (f) [116].
In an attempt to measure the overall binding affinities, Springsteen and Wang developed a method based on the competitive displacement of the fluorescent diol-containing ARS. Their findings are summarized in Table 3, under Method 2. Even though there is a lot of variability in the absolute magnitude of the reported binding constants between the two different methods, the observed trends are consistent.

The order of the affinities is determined by the relative position and orientation of the hydroxyl groups in the diol [26]. As the stability of the ester is determined by the amount of strain introduced upon binding of the borate to the diol, the sequence of stabilities should follow the degree of preorganization of the starting diols [30]. Indeed, both studies report that catechol and ARS have the highest binding constants. The rigidity of the coplanar vicinal cis-diols in these molecules (highlighted in blue in Table 3) was suggested to account for their enhanced reactivity, as the loss of configurational entropy suffered upon complexation is lower than that of ligands with additional degrees of freedom [41].

A different trend is observed with the acyclic sugar alcohols. Looking at ethylene glycol, glycerol and mannitol in Table 3 reveals how their binding affinity increases with their number of hydroxyl groups, because statistically the possibilities of binding to a boronic acid increase with the number of diols. However, this entropic contribution is not enough to account for the huge increase in stability observed by Lorand and Edwards going from glycerol (19.7 M⁻¹) to mannitol (2275 M⁻¹). Possible explanations for this include a chelating effect in the stepwise hydrolysis of the ester bond and stabilization due to intramolecular hydrogen bonding [45].

The studies also reported large differences in the stability constants of different monosaccharides. For instance, Lorand and Edwards showed that the binding constant of fructose (4370 M⁻¹) is much higher than that of glucose (110 M⁻¹) and galactose (276 M⁻¹). To account for this, the different forms of the saccharides in aqueous solution have to be considered. Table 3 shows the equilibrium between the pyranose (left) and furanose (right) forms of glucose, galactose, and fructose. Studies have shown that the most stable boronic acid-diol complexes are formed with the conformationally locked syn-periplanar hydroxyl groups on furanose rings (highlighted in blue in Table 3), while the more disorganized pyranose diols have much lower binding affinities [46,47]. Therefore, the saccharides with a larger relative percentage of

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Structure</th>
<th>Method 1 (K_{tet} (M^{-1}))</th>
<th>Method 2 (K_{eq} (M^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
<td><img src="image" alt="Structure" /></td>
<td>2.76</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td><img src="image" alt="Structure" /></td>
<td>19.7</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td><img src="image" alt="Structure" /></td>
<td>2275</td>
<td>120</td>
</tr>
<tr>
<td>Catechol</td>
<td><img src="image" alt="Structure" /></td>
<td>17500</td>
<td>830</td>
</tr>
<tr>
<td>Alizarin Red S.</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
<td>1300</td>
</tr>
<tr>
<td>D-glucose</td>
<td><img src="image" alt="Structure" /></td>
<td>110</td>
<td>4.6</td>
</tr>
<tr>
<td>D-galactose</td>
<td><img src="image" alt="Structure" /></td>
<td>276</td>
<td>15</td>
</tr>
<tr>
<td>D-fructose</td>
<td><img src="image" alt="Structure" /></td>
<td>4370</td>
<td>160</td>
</tr>
<tr>
<td>Lactose</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
<td>1.6</td>
</tr>
</tbody>
</table>
furanose in their natural speciation should have the higher stability constants. This is exactly what was observed: the composition of furanose in solution is 0.14% for glucose, followed by 2.5% for galactose and 25% for fructose [48]. Furthermore, the disaccharide lactose, which can only exist in the pyranose form, was found to have a lower binding affinity (1.6 M⁻¹) than its components glucose (4.6 M⁻¹) and galactose (15 M⁻¹).

The chemical details of boronic ester formation and breakage are complicated and remain an area of active research. Nonetheless, robust characterization of the $p_K$ of different boronic acids as well as the binding affinities for common boronic acid-diol pairs provides the user with sufficient guidelines to predict boronic ester behavior as cross-links in polymer networks. While the field will benefit from new chemistries and additional characterization of boronic ester binding kinetics and thermodynamics, the tools already exist to form materials from dynamic covalent boronic ester bonds. Control over materials properties in these dynamic covalent networks requires an understanding of the binding partners as well as how network formation and connectivity dictate macroscale properties. Engineering principles for network formation are discussed in the following section.

3. Engineering networks with boronic ester bonds

As mentioned above, dynamic covalent chemistries, including boronic ester bonds, enable the formation of responsive and moldable materials as the bonds can rearrange by breaking and reforming upon application of external stimuli on experimental timescales [43]. The main considerations in the design of these adaptable polymer networks from dynamic covalent chemistries are the specific chemistry of the binding pair and the mechanism of network formation. The specific boronic acid-diol chemistry defines the thermodynamics and kinetics of boronic ester bond formation, breaking, and reformation, which controls the dynamics of network strand rearrangement. The mechanism of network formation governs the topology of the network or how the network strands are linked together, which in turn influences the mechanical properties (such as gel point, plateau modulus, relaxation time, and swelling) of the resultant viscoelastic material. Simply put, the properties of polymer networks and gels are controlled by how the molecules in the network are connected [49]. Broad use of dynamic covalent networks and gels requires a robust understanding of how these factors influence emergent network properties. In this section, we discuss how network formation and topology relate to the mechanical properties of dynamic covalent networks.

3.1. Network formation and gel point

Polymer network formation occurs by cross-linking multifunctional monomer or macromer precursors [50]. Here, a functional group refers to a chemical moiety that can participate in the formation of a single dynamic covalent bond. Boronic ester cross-linking occurs via complementary bonding between boronic acid and diol moieties, as shown in Fig. 4. If each precursor molecule has a functionality of exactly two, the reaction will lead to chain extension and growth of individual polymer chains. On the other hand, if the average functionality is greater than two, branching can occur which bonds polymer chains together. At a critical extent of reaction $p_c$, the cross-linking of disparate polymer chains through the branch points induces a phase transition from liquid-like behavior to solid-like behavior as a three-dimensional polymer network percolates across the entire system [50]. The critical point $p_c$ is referred to as the gel point. Just below the gel point, the system exists as a solution of polydisperse branched polymers, and beyond the gel point, at least some fraction of the system exists as an ‘infinite’ molecular weight polymer network that spans the entire volume. The gel point can be calculated based on the functionality and structure of the network forming precursors, using mean-field models of gelation via the Flory [51] or Flory-Stockmayer [52,53] approaches that calculate $p_c$ as the conversion at which the weight-average molecular weight of the growing, branched polymer chains diverges.

To exploit the reversible nature of the boronic ester bond to form dynamic covalent networks, boronic esters moieties need to be installed into the backbone of the forming polymer network. Two main mechanisms can be employed to introduce boronic ester bonds into the network backbone [4]. First, networks can be formed via direct cross-linking of multifunctional boronic acid and diol monomers or macromers, as shown in Fig. 4a, b, and c. In this manner, the cross-links themselves are composed of reversible and dynamic boronic esters during formation. Alternatively, networks can be formed from monomers or macromers that contain one or multiple boronic esters in their core and pendant polymerizable functional groups (such as (meth)acrylates, thiols and alkynes), as shown in Fig. 4d. Here, the cross-linking chemistry and conditions must maintain the integrity of the boronic ester bond. As most boronic esters are highly dynamic in an aqueous solution at standard pH [117], the former mechanism that uses the boronic ester as the cross-linking chemistry itself is more common for the formation of boronic ester–based hydrogels and will be the focus of the rest of this section.

In covalently cross-linked networks and gels, a formed bond is permanent, and calculation of the gel point is relatively straightforward. For example, the critical extent of reaction $p_c$ based on the Flory approach can be calculated for multifunctional precursors as:

$$p_c = \frac{1}{f - 1}$$

where $f$ is the average functionality of the precursors [51,54]. In the Flory-Stockmayer approach, the critical extent of reaction $p_c$ for a step-growth polymerization between precursor A with functionality $f_A$ and precursor B with functionality $f_B$ can be calculated as:

$$p_c = \frac{1}{\sqrt{r(f_A - 1)f_B - 1}}$$

where $r$ is the stoichiometric ratio between A and B with $r \in [0, 1]$ [53]. Note, the two approaches converge for $r = 1$ and $f_A = f_B$.

For dynamic covalent bonds, such as boronic esters, the reacted bonds are not permanent; the bonds are in flux as they continually rearrange. Therefore, gelation in dynamic covalent networks relates also to the equilibrium constant $K_{eq}$ and the concentration of the functional groups $[C]$. At sufficiently long times after mixing, one can assume that the system has reached thermodynamic equilibrium and that the number of formed bonds or ‘extent of reaction’ is determined solely by the equilibrium constant for the dynamic covalent chemistry and the concentration of functional groups. Thus, the number of formed bonds $p = fK_{eq}[C]$ must remain above $p_c$ to maintain a gel. As discussed above, $K_{eq}$ for boronic ester bonds varies with changes in environmental factors, including pH, temperature, or stress. Modulating $K_{eq}$ provides a facile handle to reverse gelation in dynamic covalent networks and enables easy processing of the networks. External stimuli can transform the network from a solid to a liquid and back again repeatedly, allowing for recycling, healing, as well as shaping and reforming through subtle shifts in the external conditions.

A thorough treatment of the gel point for associating polymers was developed by Semenov and Rubinstein [55]. The guidelines here provide descriptive indicators for what systems will or will not
form a gel and how external parameters can be used to transition between sol and gel, reversibly. This understanding has enabled the design and synthesis of multifunctional precursors that have been used to engineer responsive materials exploiting the dynamic covalent nature of boronic ester bonds [43]. This has been demonstrated in the design of moldable materials from borax and polysaccharides, glucose-responsive gels, and self-healing polymer networks that will be discussed further below.

**Fig. 4. Formation mechanisms for dynamic covalent networks.** The choice of network formation and network connectivity, in cooperation with the selected dynamic covalent chemistry, influence macroscale properties of boronic ester-based networks and gels. In all cases, the reversible complexation between boronic acid (red) and diol (blue) forms dynamic covalent boronic esters (orange). Several strategies exist to form networks and gels using this cross-linking motif. (a) Multifunctional small boronic acid molecules can be used to cross-link polyvalent polymer chains, as exemplified by the gel formed by mixing borax and poly(vinyl alcohol). (b) Gels can also be formed by mixing polyvalent linear polymers whose backbones have been functionalized with diols and boronic acids. (c) In addition, dynamic covalent networks have been formed through the cross-linking of multi-arm star polymers. (d) Moldable networks can also be formed by cross-linking monomers that contain a previously formed boronic ester using an orthogonal pendant reactive group (purple). In all cases, the molecular weight of the polymers, the number of functional groups, the polymer concentration, and the stoichiometry between boronic acids and diols can be used to modulate bulk mechanical properties such as plateau modulus $G_0$ and relaxation time $\tau_{r}$. 

![Diagram](image-url)
3.2. Dynamic mechanical analysis

Engineering properties of major interest in the design of polymeric materials, including dynamic covalent networks, are the plateau modulus and the relaxation time. The modulus of elasticity $E$ for a material defines the ratio between the stress applied to a material and the resultant deformation in the linear elastic region. The relaxation time $\tau_r$ characterizes the timescale within which strain-induced stresses within the material are dissipated [50]. A standard technique for quantifying the linear mechanical properties of viscoelastic materials is to measure the stress in the material in response to small sinusoidal deformations. The application of cyclic strain, called dynamic mechanical analysis, is employed commonly for soft materials ($E \approx 10^1$–$10^3$ Pa) characterization using a shear rheometer, which measures the shear modulus $G$ of the material, or the ratio of shear stress to shear strain. $G$ and $E$ are related via the Poisson’s ratio [56].

Shear rheometry probes materials properties with an applied oscillatory shear strain. It measures the frequency-dependent dynamic or complex modulus $G'$ of the material, which can be expressed in terms of the storage modulus $G' (\omega)$—a measure of the stored elastic energy—and the loss modulus $G'' (\omega)$—a representation of the viscous energy dissipation [56]. Tuning the frequency of the applied strain enables investigation of different timescales or relaxation modes within the material. Owing to the many hierarchical length scales present in polymeric materials that are inherently coupled to different timescales of molecular motion, mechanical properties are dependent on the frequency of analysis. High frequency measurements probe short times and thus small motions at the atom or monomer level while low frequency measurements probe long times and thus large motions at the chain or material level. As such, the frequency-dependent modulus of all polymeric materials increases at high frequency, indicative of the glassy dynamics associated with relaxation on the smallest length scales [4]. At lower frequencies, polymer segments and chains are able to relax, and the frequency-dependent modulus decreases to a plateau modulus $G_0'$. Both the plateau modulus and the glassy region are illustrated in Fig. 5a, which plots the frequency-dependent storage modulus $G' (\omega)$ for model polymer networks.

Classic polymeric materials are subdivided into thermoplastics—polymer melts that flow with increasing temperature or dissolve upon addition of a good solvent—and thermosets—covalently cross-linked networks that do not change state with temperature prior to combustion and swell without dissolving upon addition of a good solvent. In thermosets, the topology of the network strands is fixed by the permanent covalent cross-links. These can fluctuate in three-dimensional space but prevent flow or dissolution of the network. In thermoplastics, the topology of the polymer strands is constrained by other polymer chains via physical interactions, entanglements, and excluded volume; however, at sufficiently long times, chains can overcome these physical constraints and move or reorganize to relax internal stress.

Therefore, as their network topology is fixed, thermosets behave as ideal, elastic materials. They possess a plateau modulus associated with the cross-linking density of the material and a non-finite relaxation time. A representative curve for their ideal behavior is shown in Fig. 5a. Thermoplastics, on the other hand, also demonstrate a plateau modulus, as polymer diffusion is constrained by topological entanglements with other chains that act as effective cross-links at certain timescales. However, with decreasing frequency or longer timescales, the frequency-dependent modulus decays as the polymer chains are able to reorganize via curvature diffusion, and thus thermoplastics possess a finite relaxation time, as shown in Fig. 5a.

Viscoelastic dynamic covalent networks exhibit rheological behavior that mirrors aspects of both thermosets and thermoplastics. Their behavior is characterized by the Maxwell model of viscoelasticity, which combines in series an elastic element with modulus $G_M$ and a viscous element with viscosity $\eta_M$ [50]. In the Maxwell model, a critical timescale is defined as $\tau_\infty = \eta_M/G_M$. The material will behave as a solid at timescales shorter than $\tau_\infty$ and as a viscous liquid on timescales longer than $\tau_\infty$. That is, dynamic covalent networks possess a plateau storage modulus defined by the network cross-link density as well as a finite relaxation time defined by the chemistry of the dynamic covalent cross-links, as illustrated in Fig. 5a. A complete treatment of the polymer physics and scaling laws for the behavior of reversible polymer networks was developed by Rubinstein and Semenov, and we refer the reader to these studies for a more thorough discussion of the theoretical foundations of associating polymer networks [57]. Here, we restrict our discussion to general engineering design criteria for the design of dynamic covalent networks.

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Fig. 5. Rheology of dynamic covalent networks. Dynamic mechanical analysis is used to assess the frequency-dependent properties of soft materials. (a) Traditional thermosets (black line) exhibit a plateau modulus $G_0'$ dictated by the cross-linking density with increasing modulus at high frequency associated with glassy dynamics at short length scales. Traditional thermoplastics (red line) exhibit similar behavior except at low frequency where a decrease in modulus is observed associated with network strand rearrangement via curvilinear diffusion or reptation at long timescales. Dynamic covalent networks (red dotted and red dashed lines) exhibit glassy dynamics at high frequency, a plateau modulus at intermediate frequency, and decreasing modulus at low frequency associated with network rearrangement as bonds break and reform on long timescales. In addition, for condensation reactions such as boronate ester formation, the plateau modulus decreases with decreasing $K_{eq}$. (b) Dynamic covalent networks also exhibit network relaxation on experimental timescales with a corresponding relaxation time $\tau_r$. The relaxation time is associated with the crossover frequency $\omega_\infty$; below $\omega_\infty$, the material behaves as a viscoelastic solid. The crossover frequency shifts to longer timescales with decreasing $K_{eq}$. Furthermore, the crossover point can also be influenced by the exact magnitudes of the forward and backward rate constants $k_f$ and $k_b$. 
3.3. Plateau modulus

As previously discussed, the elastic modulus is a critical parameter in the design of soft materials. The theory of rubber elasticity for cross-linked polymer networks relates the molecular details and topology of the network to the modulus of the material [50]. At its core, rubber elasticity states that \( E \sim nT \), where \( n \) is the number density of network strands and \( T \) is the temperature of the material. The number density of network strands is directly related to the cross-link density \( \rho_n \) and further depends on the molecular properties of the network precursors, e.g., molecular weight, persistence length, and concentration. The recursive method of Miller and Macosko is commonly used to calculate the number density of network strands based on the properties of the precursor molecules [58].

The molecular foundation of rubber elasticity is the entropic penalty caused by deforming individual polymer chains away from their relaxed state, which leads to a restoring force of \( k_BT \) per network strand. Here, \( k_B \) is the Boltzmann constant and \( T \) is the temperature. Thus, the modulus scales linearly with the number density of network strands with each strand contributing \( k_BT \) to the modulus. Based on rubber elasticity, the shear modulus can be calculated directly as \( G = k_BT \). Thus, the plateau modulus from shear rheometry \( G' \) can be related to the number density of network strands in the cross-linked material. Rubber elasticity is based on the underlying assumptions that the network strands are Gaussian, and the cross-links are points in free space [50]. The model stated above, \( G = k_BT \), is based on the affine network model, which assumes that each network strand experiences the same deformation as the bulk material. The phantom network model accounts for the fact that the cross-links are not fixed in space but fluctuate around an average location. This leads to an overall decrease of the predicted modulus of the material as given by \( G = k_BT (1-2f) \), where \( f \) is the functionality at the cross-link junctions. Recently, more advanced versions of rubber elasticity have been developed that account for more complicated network topology that includes network loops and entanglements including the real elastic network theory or RENT [49]. In all models, the general scaling that \( G \sim E \sim nT \) holds is inherent to the thermoelastic nature of rubbery materials.

As alluded to above, several design elements provide control over the network strand density and thus the elasticity in polymer networks and gels. Consider the step-growth polymerization of 4-arm star-polymers functionalized with boronic acid or diol derivatives, as depicted in Fig. 4c. Here, the network strand density can be tuned by altering the molecular weight of the individual polymer molecules and thereby the molecular weight of the individual arms. Additionally, the overall polymer concentration will affect the density of network strands, as well as the equilibrium bound fraction and the extent of network defects. Selecting backbone polymers with different rigidities or extents of hydration will also influence the modulus for a fixed polymer concentration. The network connectivity, or how many network strands link together at each cross-linking junction, also changes the density of active network strands for a given polymer concentration and can be increased by interchanging the 4-arm star-polymers for 8-arm star-polymers or polyfunctional linear polymers.

Uniquely, dynamic covalent networks also provide control over \( K_{eq} \) and thus the effective number of intact network strands. This affects both the gel point as described above but also the plateau storage modulus for dynamic addition and condensation networks such as boronic ester networks and gels. Some fraction of the boronic acid and diol functionalities exist in the non-bonded state, and this fraction is dictated by the environmental conditions. As \( K_{eq} \) decreases, the network plateau modulus decreases, when all other factors are kept constant, as shown in Fig. 5a. The physical properties of reversible associating networks, which include dynamic covalent networks, have been explored theoretically by several groups. Green and Tobolsky proposed an early molecular theory of the physical properties of associating and viscoelastic polymer media [10]. These initial efforts have been continued by Wang and others to develop extended theories for the behavior of transient networks [59]. Leibler et al. presented a model for the dynamics of networks composed of linear polymers with reversible cross-links [60]. Semenov and Rubinstein provided an understanding of the statics and dynamics in thermoreversible gelation in associative polymers [55,57]. Recent work from Tang et al. has adapted the phantom network model to predict plateau storage modulus for dynamic covalent networks that accounts for \( K_{eq} \) and thus the fraction of formed cross-links [61]. Building upon these directions, additional frameworks are needed to precisely link network topology and binding constants to network mechanics.

3.4. Relaxation time

The time-dependent viscoelastic properties of dynamic covalent networks can also be engineered by the cross-linking chemistry and network topology. As discussed above, these materials exhibit frequency-dependent storage \( G'(\omega) \) and loss \( G''(\omega) \) moduli, owing to the breaking and reformation of boronic ester bonds that occurs on experimental timescales. At high frequencies or short timescales, their properties are analogous to an elastic network: at low frequencies or long timescales, the material behaves like a viscous polymer solution. Oscillatory shear rheometry across a range of frequencies is used to quantify the viscoelastic behavior of dynamic covalent networks. In general, a critical frequency \( \omega_c \) is observed when \( G'(\omega) = G''(\omega) \), which is related to the relaxation time \( \tau_c \) of the material, \( \tau_c = 2\pi/\omega_c \). At frequencies below \( \omega_c \), the viscoelastic moduli should follow the classic scaling rules for terminal relaxation in a Maxwell model, where \( G'(\omega) \sim \omega^2 \) and \( G''(\omega) \sim \omega \) [57]. Fig. 5b illustrates these behaviors and the effect of varying \( K_{eq} \) or the magnitude of the forward and reverse rate constants.

The characteristic timescale of the viscoelastic behavior in dynamic covalent networks and gels is controlled by the dynamic covalent chemistry that comprises the reversible cross-links, which ultimately dictates the timescale of relaxation of the network strands. The timescale of the individual bond \( \tau_B \) is related to the relaxation time \( \tau_c \) of the network. Tuning the specific chemistry of the boronic acid-diol pair, the environmental conditions and the network topology provide control over the time-dependent properties for this class of materials [57,62]. For example, bonds with short \( \tau_B \) timescales produce materials that behave as viscous polymer melts whereas bonds with high \( \tau_B \) generate materials that can be indistinguishable from covalent networks [63].

In practice, dynamic covalent networks are not characterized by a single relaxation time but display a distribution of relaxation times. This is related both to the variance in dynamic covalent bond properties and cross-link topology in the network. The distribution of relaxation times can be determined by converting the frequency-dependent storage and loss moduli calculated from rheometry into a relaxation spectrum \( H(\tau) \), as described by Grindy et al. for metal ligand—based dynamic polymer networks [64]. The relaxation spectrum models the material as an infinite number of Maxwell models in parallel rather than the single Maxwell model mentioned previously. It can be interpreted as how much energy is stored or dissipated by the material during dynamic loading [64]. A more complete understanding of how network topology and binding thermodynamics and kinetics control the relaxation behavior in dynamic covalent networks and gels is a focus of current research.
and will be necessary to provide a priori design of materials with precise relaxation behaviors for targeted applications.

3.5. Materials assembly from boronic ester cross-linking

As described above, the reversible nature of the boronic ester bond can be exploited to build dynamic covalent networks and gels with time-dependent viscoelastic properties that are moldable and self-healing. Excellent reviews of boronic ester-based materials have been published, and we direct the reader to these works for a more comprehensive discussion of boronic ester–based materials design [43,65]. Here, we restrict our discussion first to some of the foundational work that demonstrates how boronic acid–diol chemistry and network topology can be used to engineer dynamic covalent networks from boronic ester bonds and then highlight some of the emerging biomedical applications of boronic ester–based materials.

The earliest examples of engineering materials with boronic esters involved the combination of borax with polyhydroxy compounds. Deuel and Neukom demonstrated in their seminal paper that borax could be used in aqueous solution to cross-link gels with suitable hydroxyl functional polysaccharides, including poly(vinyl alcohol) (PVA) [66]. The cross-linking of PVA with borax (Fig. 4a) is now commonly used to make moldable and self-healing ‘slime’ based on these original recipes. In their original article, Deuel and Neukom already demonstrated that materials properties depend on pH, providing evidence for an ‘optimal’ pH for gel formation and highlighted that the addition of small molecule diols, including fructose, glycerin, and sucrose, influenced materials properties by disrupting the formed cross-links. This work also explored altering the ratio of borax to polyhydroxy, which indicated that the materials properties depend on the extent of cross-linking. As a whole, this manuscript laid the foundation for the myriad of boronic ester–based materials that are now made and how they can be engineered based on their cross-linking chemistry, environmental factors, and network topology.

Building upon these concepts, Pezron et al. investigated the properties of reversible gels formed from borax and galactomannan, guaran, and (hydroxypropyl)guaran [67,68]. In these studies, 11B NMR was used to probe the extent of free borax as compared to mono-diol and di-diol complexation, providing insights into the molecular structure of the gels and the thermodynamics of binding. This work was extended by Leibler et al. to construct physical models of the properties and dynamics of reversible networks based on the underlying structure and chemistry [60]. Building on these findings, Kesavan and Prud’homme studied the linear viscoelastic properties of guaran and (hydroxypropyl)guaran, corroborating with these data the theory developed by Leibler et al. for the rheology of reversible networks [69].

A major focus for the modern use of boronic ester–based materials has been on the sensing of carbohydrates. Kitano et al. incorporated PBA moieties into poly(N-vinyl-2-pyrrolidone) [poly(NVP-co-PBA)], which was combined with PVA to assemble glucose-responsive gels. These responded to glucose by undergoing a gel-sol transition, envisioning applications as glucose-responsive drug delivery platforms [70]. In this work, they demonstrated the influence of polymer molecular weight, stoichiometry, and diol chemistry on materials rheology. The general concept of using boronic ester bonds for the assembly of materials that respond to the presence of carbohydrates has been explored by many other groups. For example, Kataoka et al. engineered a range of glucose-sensing materials from PBA-based materials [71,72].

An additional emphasis in the community has been on the general design of moldable and self-healing networks and gels using the dynamic covalent boronic ester bond. Roberts et al. synthesized linear polymers of acrylic acid or 2-hydroxypropylmethacrylamide containing 10 mol% of PBA or salicylhydroxamic acid (SHA) [73]. Upon mixing in aqueous solutions, the PBA and SHA containing polymers assembled to form dynamically restructuring hydrogel networks, best illustrated by the scheme in Fig. 4b, whose properties were tuned by the volume fraction of polymer and pH. Similarly, as depicted in Fig. 4c, He et al. assembled reversible hydrogels from catechol-functionalized 4-arm poly(ethylene glycol) (PEG) and 1,3-benzenediboronic acid, exploiting the high affinity binding between PBA and catechol to engineer pH-responsive and self-healing gels [74]. In this work, they demonstrated that higher concentrations of the multi-arm PEG increased the plateau modulus of the formed network. Cash et al. installed dynamic covalent boronic ester bonds directly into polymer networks through the direct thiol–ene photopolymerization of 4-[(allyloxy)methyl]-2-(4-vinylphenyl)-1,3,2-di-oxaborolane, a boronic ester containing divinyl monomers, with multifunctional small molecule thiols [75]. The installation of the reversible boronic ester bond into the network backbone during photopolymerization delivered tough and self-healing polymer networks, as illustrated in Fig. 4d. In an analogous approach, Theato et al. built moldable and self-healing gels by incorporating borax into the monomer mixture during network formation via Michael-type addition of PEG diacrylate and dithiobetitol, a 1,2-diol-containing dithiol [76]. Cromwell et al. designed dynamic networks through the chemical synthesis of 1,2-diol-containing poly(cyclooctene and diphenylboronic ester linkers [15]. In this manner, they fabricated solvent-free polymer networks that were self-healing and reprocessable, with the self-healing rate controlled by the small molecule PBA-diol binding kinetics.

Advancing from these early demonstrations, Yesilyurt et al. fabricated dynamic covalent networks from 4-arm PEG molecules functionalized with 1,2-diols or PBA derivatives (Fig. 4c), which highlighted the ability to tune materials properties with boronic acid–diol chemistry and network topology [7,77]. In these well-defined networks, they explored the influence of altering the boronic acid chemistry, which modified the optimal pH of network formation and the crossover frequency (ωc) [7]. In addition, they showed that tuning the weight percent of polymer controlled the plateau modulus but did not influence significantly the relaxation time of the gel [77]. Network dissolution and release of entrapped molecules was modulated by the presence of free diols, including glucose, and the gels were designed as injectable materials based on their shear-thinning and self-healing properties. In total, the studies presented here on the design of dynamic covalent networks and gels from boronic ester bonds illustrate that control of materials properties is enabled by tailoring both the chemistry of the reversible bond and the network topology.

4. Emerging biomedical applications of boronic ester–based materials

Traditional polymeric materials, based on thermosets and thermoplastics, are designed with a specific set of properties for a given application and lifetime. A major force in modern materials science research is the synthesis of dynamic materials with functions beyond those of static polymeric materials [78]. As we transition from static to dynamic materials design, we envision ‘intelligent’ systems that can respond to external stimuli and interact with the surrounding environment [79,80]. Self-assembly and supramolecular chemistry are both robust synthetic tools for the fabrication of dynamic soft matter [81,82]. Within this framework, the use of dynamic covalent bonds, such as boronic ester bonds, provides a versatile approach to assemble responsive networks and gels. The unique ability of dynamic covalent bonds to
break and reform in response to external stimuli enables the design of polymeric systems that exhibit macroscale changes to select environmental factors [78]. Responsive polymeric materials composed of boronic ester dynamic bonds have been developed exploiting this approach, with a particular emphasis on self-healing networks and carbohydrate-responsive gels. Traditional applications of boronic ester–based materials, discussed briefly above, have been reviewed in depth elsewhere [43,65]. Here, we present recent efforts in the emerging areas enabled by insights into the boronic acid–diol binding chemistry, advances in polymer design, and new strategies for network formation. Specifically, we highlight the use of boronic ester-based materials as responsive drug delivery systems (DDSs), dynamic scaffolds for cell culture, and engineered adhesives.

4.1. Responsive drug delivery systems

New therapeutic molecules to treat disease are under constant development. While each molecule is developed to modulate the underlying biology and restore homeostasis in vivo, the efficacy of a drug is dependent critically on the manner by which it is administered in the body [86]. Complications can arise if the molecule is present at the wrong time, the wrong concentration, or at the wrong site. In addition, many emerging therapeutics are comprised of poorly soluble hydrophobic small molecules or complex biologics, such as nucleic acids, antibodies, and protein conjugates, which require a material carrier for stability and efficient delivery in vivo [87]. Therefore, an attractive application of ‘intelligent’ materials based on dynamic boronic ester bonds is for the design of responsive DDSs that can control the release of emerging and existing therapeutics. In the future, this could include the design of injectable materials that can be applied in a minimally invasive manner at the target site, sense aberrant biological signals, and respond by releasing corrective therapeutics [80].

Initial uses of reversible boronic ester bonds for drug delivery focused on direct modification of therapeutics or delivery vehicles with boronic acid or diol moieties. As an early example, Shino et al. engineered PBA-functionalized gel beads for the design of diol binding chromatography columns [88]. They then functionalized insulin with gluconic acid (with ~2 gluconic acid residues per insulin molecule) which could be loaded onto the PBA gel bead column. Toward the development of an autoregulated insulin pump, the release of insulin was triggered in response to changes in glucose levels. In a similar approach, Su et al. designed a pH-responsive polymer conjugate of the FDA-approved anticancer drug bortezomib (BTZ) [89]. In this study, a catechol-functionalized PEG molecule was synthesized and bound to BTZ to produce the polymer conjugate. The reversible nature of the boronic ester bond was exploited to release BTZ in acidic environments, e.g., tumor microenvironment or subcellular endosome. Wang et al. extended the conjugation approach to design PBA-functionalized chitosan nanoparticles (CS-PBA-NPs) to bias the biodistribution of doxorubicin-loaded NPs to sialic acid residues present on liver cancer cells [90]. CS-PBA-NPs were synthesized with cell pene-trating and adhesive (RGD) peptides for improved uptake. While complex in their design, this system demonstrated the utility of exploiting boronic ester binding to increase the residence time and uptake at a target site.

Beyond conjugation approaches, several groups have investigated the use of rationally engineered responsive gels for the controlled release of molecular therapeutics. Matsumoto et al. fabricated hydrogels based on poly(N-isopropyl-methylacrylamide) and 4-(2-acrylamidoethylcarbamoyl)-3-fluorophenylboronic acid that were designed to swell in the presence of elevated glucose levels (hyperglycemia) [83]. The principle was based on the creation of phenylboronate anions upon complexation of the PBA with glucose, which induced a volume change and corresponding increase in mesh size of the gel. As shown in Fig. 6a, when the glucose concentration was kept low (normoglycemia), a localized dehydration layer (‘skin layer’) formed on the outer surface of the gel, enabling control of the release of insulin from the gel. This system demonstrated pulsatile, glucose-responsive release of insulin in vitro using fluctuations between hyperglycemic and normoglycemic conditions. In another approach to design glucose-responsive insulin release, Zhang et al. assembled layer-by-layer films exploiting boronic ester bonding between PVA and poly [acylamide-co-3-(acylamide)-phenylboronic acid] [91]. Insulin was incorporated into the PVA layers, and film dissolution, including insulin release, was accelerated by the presence of glucose.

Another emerging use of the reversible nature of boronic ester bonds is the design of injectable biomaterials for controlled drug delivery. As the boronic ester bonds can break and reform, gels with shear-thinning and self-healing properties can be assembled using these dynamic covalent bonds as cross-links. Dong et al. assembled injectable hydrogels using an acrylamide copolymer containing boronic acid and glucose functionalities [92]. Owing to the complexation between boronic acid and glucose derivatives on disparate chains, the solution of copolymer formed a gel, and this material exhibited shear-thinning and self-healing properties. In addition, a model therapeutic was released in response to the addition of free glucose. This approach was extended by Yesilyurt et al. to form defined hydrogel networks from PBA and diol functionalized 4-arm PEG molecules [7]. These gels also exploited reversible boronic ester bond formation as a cross-linking chemistry, and the gels exhibited shear-thinning and self-healing properties that enabled injection through standard gauge syringe needles. Again, release of model therapeutics was accelerated in glucose containing aqueous solutions. Recently, Huang et al. presented another use of dynamic boronic ester bonds to fabricate injectable DDS [93]. Here, the bioactive polyphenol, containing two functional diols, was used to form cross-links with boronic acid–functionalized multi-arm PEG molecules. In this manner, the material was injectable owing to the responsive boronic ester bonds and released the bioactive polyphenol as the gel dissolved in aqueous media.

These recent examples using reversible boronic ester bonds to control the release of therapeutic agents highlight the ability of dynamic covalent bonds to engineer ‘intelligent’ DDS. Special emphasis in the use of boronic ester bonds has been centered on the design of glucose-responsive DDS. It should be noted that as the binding between the PBA and glucose is relatively weak, the release in many of the glucose-responsive materials is not much faster than in aqueous buffer alone. Other biological triggers beyond glucose, such as pH or fructose, may provide more specific release from boronic ester-based responsive gels.

4.2. Dynamic scaffolds for cell culture and tissue engineering

As we learn more about the dynamic nature of the native extracellular matrix, increased emphasis has been placed on the design of synthetic mimics that enable temporal modulation of biophysical and biochemical cues in the model cell niche [94]. Engineered hydrogel platforms have advanced 3D cell culture and tissue engineering, and a current focus in biomaterials is to move from static niches to dynamic and user-controlled niches [79,95]. Increasingly, dynamic niches reveal critical aspects of biology and development that were not observable in static materials. For example, it has been shown that the biophysical properties of the cell niche influence cell function, including differentiation [96–98].
Recent research with responsive culture scaffolds has highlighted that dynamic physical properties of the cell niche also influence cell fate and function [99–101].

Initial demonstrations of the utility of boronic ester bonds in the presence of living cells were focused on surface engineering to modulate cell attachment. Aoki et al. generated acrylamide-based polymer surfaces that were functionalized with PBA for the culture of bovine aortic endothelial cells (BAEC) [102]. BAECs adhered to the acrylamide surface without the need for additional protein coating, presumably through interactions between surface-bound PBA moieties and cell surface carbohydrates, and the cells formed model capillary structures. In another demonstration of surface functionalization for control of cell attachment, Liu et al. grafted a poly(acrylamidophenylboronic acid) brush from a silicon nanowire surface array [103]. The engineered, PBA-presenting surface was exploited to selectively capture cancer cells that overexpress sialic acid on the cell surface. When the ‘blocks’ are joined, self-healing of the material occurs, enabling 3D co-culture of the two different cell types.

The ability to assemble dynamic 3D materials from boronic ester bonds has also been exploited for the direct encapsulation of mammalian cells. Konno et al. synthesized boronic acid containing phospholipid polymers, which formed gels upon mixing with diol containing polymers including PVA [104]. This approach was used for the reversible encapsulation of murine fibroblast cells, demonstrating a general approach for the 3D encapsulation and release of mammalian cells within dynamic covalent gels. Recently, Chen et al. developed a dynamic 3D culture platform based on dynamic covalent bonds between benzoxaborole and catechol [105]. In this work, zwitterionic polymers based on methacryloyloxyethyl phosphorylcholine were synthesized with pendant catechol or benzoxaborole moieties. The benzoxaborole was chosen on account of its lower $pK_a$ (7.2), which enabled robust gel formation upon mixing of the two polymers at pH 7.4. As expected for dynamic covalent networks, the materials were shear-thinning and self-healing and enabled encapsulation and culture of cells for 24 h, which is similar to the timeframe upon which gels dissolved in pure phosphate buffered saline (PBS). Faster dissolution was observed in PBS with fructose. To extend the length of time for 3D cell culture within viscoelastic boronic ester-based hydrogels, Tang et al. synthesized gels containing dynamic boronic ester cross-links as well as permanent azide-alkyne cross-links [61]. To achieve this, the authors synthesized 8-arm PEG molecules end-
functionalyzed with both 2-fluorophenylboronic acid and azide as well as 8- arm PEG molecules end-functionalized with dibenzylcyclooctyne or nitropropamine. Upon mixing of all three components, the authors argue that a dual-network was formed with viscoelastic properties from the dynamic covalent chemistry that did not dissolve with cells in culture for up to 7 days.

The ability to reversibly tune the mechanical properties of a dynamic hydrogel scaffold could prove very useful for 3D cell culture, as cellular fate and function are influenced by the dynamic properties of the cell’s environment. To this end, Accardo and Kalow tuned the stiffness of a dynamic hydrogel by using photo-switches to control the reactivity of the dynamic covalent cross-links in the material [106]. They functionalized the ends of 4-arm PEG macromers with either azobenzene boronic acids or diols. Upon mixing, viscoelastic and stress-relaxing dynamic hydrogels were produced. Exposure to certain wavelengths of light changed the conformation of azobenzene, which in turn shifted the chemical equilibrium of the boronic acid - diol condensation. Stiffening and softening occurred as the increase/decrease in the equilibrium constant generated a higher/lower cross-linking density in the network.

Beyond 3D cell culture of single-type cell populations, Smithmyer et al. exploited boronic ester—based gels for the combined culture of multiple cell types [84]. This work leveraged the synthesis of a statistical copolymer of 90 mol % N,N-dimethylacrylamide and 10 mol % pinacol-protected 2-acrylamido phenylboronic acid to form dynamic and self-healing gels upon mixing with PVA. Uniquely, the authors demonstrated that disparate populations of cells could be encapsulated within separate gels and then assembled into a single microtissue, owing to the self-healing nature of the material. This approach, illustrated in Fig. 6b, will enable future research to investigate cell–cell interactions in 3D culture and to assemble more complicated tissue engineering constructs. In another use of reversible boronic ester gels for tissue engineering applications, Tseng et al. used dynamic covalent gels as sacrificial templates for vascular structures [107]. The gels were synthesized from polyol-containing polymers and borax and deposited in the form of the desired vascular structure. The sacrificial gel was then encapsulated in collagen or fibrin gel and subsequently removed upon addition of glucose-containing media. Engineered vessels were then developed within the resultant tubules.

There is growing interest in the use of responsive, moldable, and viscoelastic hydrogels as 3D culture platforms for cell encapsulation and tissue engineering [100]. Building upon the early studies using boronic ester—based hydrogels discussed here, we anticipate that the boronic ester binding motif will be used to engineer materials for many in vitro and in vivo cell culture applications. However, one of the main limitations of boronic ester—based gels is their lack of stability, which limits sustained use in cell culture media. New approaches that combine viscoelastic gels with covalent chemistry or incorporate other features to improve stability are needed. In addition, as the cells can rearrange the network at the cellular and subcellular length scales, characterization techniques to assess local properties (such as modulus or ligand density) are essential, as these may deviate significantly from the values measured for the bulk gel, confounding biological observations [108].

4.3. Engineered adhesives with dynamic covalent bonds

Boronic ester—based materials are also being developed for other applications in materials science outside of biomedicine. For instance, next-generation adhesives are being designed to allow materials with arbitrary surface chemistries to hold strongly to disparate surfaces with different chemistries. Traditional adhesives efficiently adhere two materials together; however, few enable stimuli responsive changes to the molecular adhesion state. Ideal adhesives would enable on-demand debonding or release of the formed adhesion through reversible bonding or efficient tuning of relative cohesive and adhesive forces. Therefore, dynamic covalent chemistry is being integrated into engineered adhesives to enable improved bonding to varied surfaces as well as to provide a handle for on-demand release. Within this approach, boronic ester bonds have been incorporated into soft matter adhesives.

In one example, Caretti et al. expanded upon standard PVA-borax gels to engineer sticky adhesives for cleaning soiled art by incorporating co-solvents, such as 1-propanol, into the solvent phase of the material [109]. With the addition of 1-propanol as a co-solvent, the gels remained moldable and could be applied at the surface of sensitive materials, including an oil painting from the 16th-17th century. Further, the addition of co-solvent modulated the polarity of the system and enabled cleaning of oxidized varnish from the painting surface. To generate self-healing materials with engineered cohesion, Cash et al. fabricated solvent-free polymer networks with boronic ester bonds in the network backbone [75]. This material was able to self-heal upon application of water at the damaged interface through the rearrangement and reformation of boronic ester bonds at the interface. In another study, reversible, wet adhesives were developed using dopamine and boronic acid functionalized polymers [85]. These demonstrated pH-responsive adhesion, as illustrated in Fig. 6c. The wet adhesive functioned at low pH due to the free dopamine groups available for interfacial binding. Adhesive properties were lost at high pH, as free dopamine moieties were consumed due to increased boronic ester complexation within the material. Stimuli-responsive adhesives were also designed using boronic acid–functionalized alginate [110]. Here, boronic ester was formed between the pendant boronic acids and diols inherent to the alginate polymer.

Adhesives are only one example of how the dynamic covalent nature of boronic esters can be exploited in soft materials design. As our understanding of how to engineer dynamic covalent networks with specific moduli, relaxation modes, and surface chemistry increases, boronic ester—based materials will likely find broad application as processable, self-healing, and stimuli-responsive soft matter. Work by Cromwell et al. and Cash et al. have convincingly demonstrated the utility of boronic ester bonds in the design of non-swollen polymer networks [15,75]. In many cases, the chemistry now exists to develop new polymer architectures which will enable materials with the desired properties for a range of applications.

5. Conclusions and outlook

The fascinating emergent properties of dynamic covalent networks based on boronic ester cross-links have led to widespread interest in this class of materials, especially within the biomedical materials community. In this review, we have highlighted the current state of the field with an emphasis on how the chemistry and network formation at the molecular scale influence materials properties. The widespread interest and use of dynamic covalent networks based on boronic ester cross-links has been enabled by insight into boronic acid – diol complexation and the polymer physics of non-covalent polymer networks. This has led to applications of these materials as responsive DDSs, dynamic scaffolds for cell culture, and as engineered adhesives. The field of dynamic covalent networks using boronic ester bonds has matured significantly in the recent years; however, we remain limited by the incomplete knowledge of several aspects of these materials.

We do not have a full understanding of boronic ester chemistry, and thus additional efforts to characterize the binding kinetics and
thermodynamics of boronic esters are needed. While some aspects of the chemistry are well-characterized, as highlighted in this review, many questions remain. What is the precise reaction landscape during boronic acid-diol complexation? How precisely do the molecular details of the cross-linking chemistry and network forming components. This remains a challenge for dynamic covalent networks; however, new rheological approaches and theoretical frameworks are being developed to provide new insights into these fundamental questions. As the field matures, we anticipate that dynamic covalent cross-links from boronic esters will continue to play a large role in the fabrication of next-generation, ‘intelligent’ soft matter. As the distinction between synthetic and living matter dissolves, dynamic covalent networks and gels will provide necessary tools to realize engineered responsive materials [80]. Design of materials that overlay multiple dynamic covalent chemistry or integrate non-covalent and covalent chemistries within a single material will enable new applications of these materials. Improved imaging and modeling will allow us to predict and understand how these responsive materials evolve on different length scales and timescales during use. There is broad potential for this class of materials, and continued interactions between polymer engineers, chemists, and bioengineers will enable new applications of these materials. Improved imaging and modeling will allow us to predict and understand how these responsive materials evolve on different length scales and timescales during use. There is broad potential for this class of materials, and continued interactions between polymer engineers, chemists, and bioengineers will enable new applications of these materials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtchem.2018.12.001.

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