Mini Review Article

Polymeric Nanoparticles and Their Novel Modifications for Targeted Delivery of Bortezomib

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Abstract
Bortezomib (BTZ) as a specific proteasome inhibitor is used to inhibit proliferation and migration of tumor cell in variety of cancers. Targeted delivery of this drug not only would minimize its unwanted side effects but also might improve its efficacy. This purpose could be gotten through different pathways but using efficient carriers may be the best one without using any additional ingredients/ materials. Some polymer based nanoparticles with specific functional groups have the ability to interact with boronic acid moiety in BTZ. This reaction might play an important role not only in cancer targeting therapy but also in loading and release properties of this drug. Novel modification such as making multifunctional or pH-sensitive nanocarriers, may also improve anticancer effect of BTZ. This review might have remarkable effect on researchers’ consideration about other possible interactions between BTZ and polymeric nanocarriers that might have great effect on its remedy pathway. It has the ability to brought bright ideas to their minds for novel amendments about other drugs and delivery systems.

Introduction
Cancer treatment strategy needs great attention because of resistant to current chemotherapy strategies and adverse side-effects on non-targeted organs.1-2 Bortezomib (BTZ) as a reversible proteasome activity inhibitor3 plays role in cancer treatment and would be targeted smartly through nanoparticles for tumors in several organs such as bone,4 breast,5 esophagus,6 cervix,7 colorectal,8 subcutaneous,9 pancreas and lung.10 Nanoparticles based on some polymers such as chitosan,11 dendrimer,12 natural polyphenols8 and other different substances were studied for delivery of BTZ (Table 1). These delivery systems would also be prepared in different forms such as micelles13 and hydrogels14 with diverse targeted delivery methods such as pH-sensitive15 and magnetic field16 applications. Designing multi-functional nanoparticles as a novel delivery strategy has also the ability to be introduced as a system for overcoming multiple drug resistance through quick action.17 These multiplexed nanoparticles attract researchers’ attentions because of their capabilities such as co-delivery of multiple therapeutic agents,18 targeting and killing diseased cells with minimal adverse effects,19 controlled release of drug20 and illustrating their location by imaging techniques and monitoring treatment in progress.21 Owing to their features, BTZ delivery by these systems were studied by some research groups to evaluate its curative performance.20-23 Through loading process of BTZ in polymeric nanoparticles some reactions would be created between this drug and nanocarrier that not only promote its therapeutic effect at tumor site but also decrease its nonspecific release.24 Both hydrophobic25-26 and ionic interactions might be responsible in this process but boronate linkage as an ionic interaction is the most common one.27-28 This interaction may be composed between boronic acid moiety in BTZ and specific functional groups such as catechol,27,29 carbonyl10 and amine9 in polymers. It is also important to say that this chemical relation have prominent influence on BTZ loading efficiency and release profile.29 This article intended to bring an attractive insight about different types of polymeric nanoparticles that had been used for targeted BTZ delivery to different tumor sites. Possible physical and chemical interactions between BTZ and polymers that might have attractive influence on its different characteristics such as loading, release, stability and potency are also mentioned. Novel BTZ delivery strategies are also other important findings that is presented in this review.

Polymeric based nanoparticles
Polymeric micelles
BTZ delivery through polymeric micellar nanostructures

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### Table 1. BTZ delivery through polymeric nanosystems.

<table>
<thead>
<tr>
<th>Nanocarrier</th>
<th>Composition</th>
<th>Size after loading</th>
<th>Cell line/animal model</th>
<th>Targeted organ</th>
<th>Remarkable findings about BTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Polymeric Micelle</strong></td>
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<tr>
<td>PAMPDA</td>
<td></td>
<td>62 nm</td>
<td>MCF7 cells</td>
<td>Breast</td>
<td>-Selective accumulation in targeted tissue.</td>
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<td></td>
<td>-Efficient cytotoxicity against tumor cells.</td>
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<td>-Long circulation time.</td>
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<td></td>
<td></td>
<td></td>
<td>-Sustained release of BTZ.</td>
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<tr>
<td></td>
<td>PEG-P(TMC-DTC)</td>
<td>48-49 nm</td>
<td>MDA-MB-231 Female balb/c mice</td>
<td>Breast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HA-CCMs</td>
<td>76-80 nm</td>
<td>Balb/C mice</td>
<td>Bone</td>
<td></td>
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<tr>
<td></td>
<td>PEG-b-P(LL-g-Cat-BTZ) and ALN-PEG-b-PLLZ</td>
<td>Dependent to polymers ratio</td>
<td>MDA-MB-231 cells/ Female balb/c mice</td>
<td>Breast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Telodendrimers (PEG5k-Cys4-LB-CA8)</td>
<td>20 nm</td>
<td>KYS30 cells</td>
<td>Esophagus</td>
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<tr>
<td></td>
<td>PEG-b-PS-b-PGAMA</td>
<td>60 nm 70 nm</td>
<td>It was not reported.</td>
<td>It was not reported.</td>
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<tr>
<td></td>
<td>PEG-b-PDEA-b-PGAMA glycopolymers</td>
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<tr>
<td></td>
<td>Chitosan coated magnetic iron oxide</td>
<td>Average core size between 5-7 nm</td>
<td>HeLa and SiHa cells/</td>
<td>Cervix</td>
<td>-Improvement cytotoxicity against tumor cells.</td>
</tr>
<tr>
<td></td>
<td>Chitosan with chondroitin sulfate</td>
<td>186.5 nm</td>
<td>HT-29 and HCT-116 cells/ female balb/c mice</td>
<td>Colorectal</td>
<td>-Sustained release of BTZ.</td>
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<td></td>
<td>Anti-CD38 chitosan NPs</td>
<td>50 nm</td>
<td>MM.1S, H929, and RPMI828/ female 7-week old SCID mice</td>
<td>Bone</td>
<td>-Reducing the frequency and amount of administered BTZ.</td>
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<td></td>
<td>G5-PPI-NH2</td>
<td>It was not reported.</td>
<td>It was not reported.</td>
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<td></td>
<td>G4-PAMAM-NH2</td>
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<td></td>
<td>Providing targeted delivery of BTZ.</td>
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<td></td>
<td>Dendrimer</td>
<td></td>
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<td>-Stability improvement at physiological condition.</td>
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<td></td>
<td>Maltose-modified hyperbranched poly(ethylene imine)</td>
<td>10 nm</td>
<td>hMSC</td>
<td>Bone</td>
<td>-Changing dendrimer behavior because of pH variation.</td>
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<td></td>
<td>G5 PAMAM</td>
<td>80 nm</td>
<td>MDA-MB-231 Female balb/c nude mice</td>
<td>Breast</td>
<td>-Solubility improvement.</td>
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<tr>
<td>Polymeric hydrogel</td>
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<td></td>
<td>-Sustained release of BTZ.</td>
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<td></td>
<td>-Have the potential for topical administration in tumor site.</td>
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<td>Natural polyphenols</td>
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<td>Tannic acid</td>
<td></td>
<td>125 nm</td>
<td>NIH 3T3 and MDA-MB-231/ mice</td>
<td>subcutaneous and bone</td>
<td>-High and controllable drug loading.</td>
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<td></td>
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<td>-Acceptable biocompatibility to normal tissues with high cytotoxicity against tumor cells.</td>
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</table>

have been studied in different cancers such as multiple myeloma, breast and esophagus. This drug showed affinity to these carrier systems by physical and chemical interactions. Physical interactions were through incorporation into the core of the polymeric micelles such as PAMPDA which was constructed from poly (N-acryloyl morpholine) block (PAM) and a hydrophobic catechol-bearing block- polydopamine (PDA) and PEG-b-PS-b-PGAMA that was prepared from poly(ethylene glycol)-block-poly(styrene)-block-poly(gluconamido ethyl methacrylate) and PEG-b-PDEA-b-PGAMA which was prepared from poly(ethylene glycol)-block-poly(2(diethylamino)ethylmethacrylate)-block-poly(gluconamido ethyl methacrylate). It is worth to say that in a study about micellar nanof ormulation based on PEG-P(TMC-DTC) (poly(ethylene glycol)-b-poly(trimethylene carbonate-co-dithiolane trimethylene carbonate)) block copolymers, physical entrapment of BTZ inside the core of micelle was because of esterification of BTZ with pinanediol. Some studies declared that boronic acid moiety in BTZ structure could interact chemically with catecholic polymers (Figure 1) such as PAMPDA, PEG-b-PGAMA and PEG-b-P(LLL-g-Cat) (poly(ethylene glycol)-block-poly(L-lysine)-graft-Catechol).
This interaction also has pH-dependent ability that lets drug release in acidic pH (almost 5) of endo/lysosomes after endocytosis by cancer cells.\textsuperscript{13,33} BTZ has also some limitations such as poor selectivity and rapid clearance from the body that may be solved by targeting strategy. Hyaluronic acid in the shell of core-disulfide-crosslinked biodegradable micelles which was obtained from poly (trimethylene carbonate-co-dithiolane trimethylene carbonate, has the ability to improve BTZ selectivity by actively targeting to the CD44-overexpressed multiple myeloma tumor in mice.\textsuperscript{25} Cyclic-RGD (cRGD) peptide (Arg-Gly-Asp), as another targeting ligand showed high affinity to MDA-MB-231 triple negative breast cancer cells that overexpressed highly with α\textsubscript{v}β\textsubscript{3} integrin. Wu et al.\textsuperscript{5} declared that cRGD-micelles showed efficient tumor accumulation in MDA-MB-231 tumor bearing mice (2.3-fold higher tumor uptake in comparison with non-targeted micelles) with less adverse effects to other tissues. Alendronate as another targeting ligand\textsuperscript{31,34} was studied because of its bone targeting property. It could be used in multi-component mixed micelles composed from poly(ethylene glycol) and poly(L-lysine) for BTZ delivery in treatment of breast cancer bone metastasis\textsuperscript{45} or multiple myeloma.\textsuperscript{34}

**Chitosan**

Chitosan as a natural, biocompatible and biodegradable polymer can be introduced as a preferable carrier in pharmaceutical fields.\textsuperscript{7,25} Delivery of BTZ by chitosan nanoparticles had been studied for multiple myeloma\textsuperscript{41} and colorectal\textsuperscript{8} cancer therapies. FTIR analysis declared that BTZ might interact with chitosan through boronic acid moiety (B–O stretching\textsuperscript{7,8} and B–O–H stretching\textsuperscript{7} of boronic acid).

BTZ instability is one of its limitations at conventional chemotherapy that reduced its bioavailability.\textsuperscript{36} Unsoy et al. research group proved that BTZ loaded in chitosan coated with magnetic iron oxide nanoparticles were quite stable after an initial release of about 15% drug in phosphate buffer solution (pH 7.4) at 37°C, and its stability did not affect by temperature variation between 37°C and 4°C.\textsuperscript{7} Chitosan carriers with folic acid and anti CD38 were also used as targeting ligands to colorectal and multiple myeloma chemotherapy, respectively.\textsuperscript{9,11}

**Dendrimer**

Dendrimers with special hydrochemical structure, has terminal functional groups with hydrophobic internal architecture that made them ideal for loading of drugs with hydrophobic characteristic like BTZ. In a study about 5.0 G of poly (propylene) imine (PPI) and 4.0 G poly (amidoamine) (PAMAM) dendrimers, Chaudhary et al.\textsuperscript{26} showed that high encapsulation capacity of G5-PPI-NH\textsubscript{2} in comparison with PAMAM-NH\textsubscript{2}, was because of more electrostatic interactions between hydrophobic moieties of PPI and BTZ. In phase solubility studies it was concluded that both of PPI and PAMAM increased solubility of BTZ more than 1000 times in comparison with plain drug (aqueous solubility 0.0532 mg/ml) and it lets dendrimers to be introduced as an ideal carrier for improving the solubility of poorly water soluble drugs.\textsuperscript{36,37} Providing a short-term retarding release of BTZ (up to 96 h) from dendritic glycopolymer PEI-Mal-B (maltose-modified hyperbranched poly (ethylene imine)) in calcium phosphate bone composites,\textsuperscript{32} demonstrated one of the advantages of dendrimers that restricts burst-release of it in chemotherapeutic processes.

Cyclic RGD-targeted dendrimers could also be used in bone tumor model which was created by injection of MDA-MB-231 cells into the tibia of mice. Fluorescent analysis documented that through targeting process, drug loaded dendrimers could be internalized by breast cancer cells and depressed the development of metastasis in an efficient manner.\textsuperscript{29}

**Other polymers**

Supramolecular hydrogel as another controllable drug delivery system for hydrophobic drugs\textsuperscript{39} was introduced for delivery of BTZ. Sustained release of BTZ from a supramolecular hydrogel in phosphate buffer solution within 12 h experimental period, had been studied by Pu et al. This nano-delivery system was composed of three catecholic peptides based on Nap-GFFY that only differ in the number of glutamic acids in their structures. They said that sustained release of BTZ was because of boronate ester hydrolysis in topical administration near tumor site.\textsuperscript{14} Natural polyphenols as abundant micronutrients in our dietary sources\textsuperscript{9} were introduced for delivery of BTZ. They have the ability of interaction with active site of BTZ through boronate ester bonds.\textsuperscript{46} Wang et al.\textsuperscript{7} deduced from this interaction and composed definite carrier consisting of catechol-containing natural polyphenols such as tannic acid (TA) and ferric ion. They used iron (III) for enhancing stability of supramolecular through making interchain iron (III)-catecholate coordination bonds and acting as a magnetic resonance system. They showed pH-dependent release of BTZ in that carrier because of boronate ester bond between polyphenols and BTZ and interchain iron (III)-catecholate coordination, that dissociated at acidic conditions in tumor microenvironment. This behavior dramatically induced BTZ remaining in MDA-MB-231 tumor beraing mice up to 9.27 % (%injected dose/gram tissue) after 24 h injection and improved its apoptosis in cancer cells.

Other copolymeric nanosystems can also be introduced for the delivery of BTZ. Shen et al.\textsuperscript{41} used a diblock copolymer composed of poly (ethylene glycol)-block-poly (D, L-lactide) (PEG-b-PLA) with amphiphilic characteristic for delivery of BTZ. This biodegradable nanoparticle made efficient uptake of BTZ by triple negative breast cancer cells (such as MDA-MB-468 cells) in comparison with plain drug (215.9 and 91.3 ng/2 × 10\textsuperscript{5} cells, respectively). Another amphiphilic polymer based on branched polyethyleneimine (PEI) and palmitic acid (PA) showed a
pH-sensitive release in in-vitro buffered solution\textsuperscript{41} which would make BTZ with less systemic toxicity and high release in acidic compartments of tumor cells.

**Novel modifications in BTZ delivery**

**Multifunctionalization**

Multifunctional nanoparticles attract researchers' attention in medical applications because of their integrated ability such as targeting, therapeutic and imaging functions in a single carrier (Figure 2). In a study by Gu \textit{et al}.\textsuperscript{23}, hyaluronic acid was used in the surface of single copolymeric micelles as a targeting ligand to CD44-overexpressed LP-1 multiple myeloma cells. Another multifunctional nanoparticle was developed with Wang \textit{et al}.\textsuperscript{20} to improve treatment efficacy in MM treatment. They co-deliver BTZ and doxorubicin through telodendrimer micelles and proved that by codelivery of the mentioned drugs, their ability in tumor growth inhibition and in treatment of H929 MM and SKOV-3 ovarian cancer cells increased. DiD as a near-infrared fluorescence was also co-loaded to evaluate biodistribution of this nanoformulation.

**pH-sensitivity**

pH-responsive manner of polymeric nanoparticles and BTZ as novel strategy, can be considered to be a promising method for optimizing loading and control release of this drug. Boronic acid group in BTZ is mainly responsible for the reaction with other moieties such as hydroxy\textsuperscript{27} or carbonyl\textsuperscript{29} groups in delivery system. Interaction sites of BTZ and polymers with pH responsive characteristics have been illustrated in Table 2. This property makes BTZ to have different loading efficiencies in different pH conditions. Demirdogen \textit{et al}. reported that BTZ have the highest and lowest loading efficiency on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) copolymer in potassium phosphate buffer at pH values ranging from 6.0 to 7.4 and in acetate buffer at pH 4.7, respectively.\textsuperscript{26} Almost the same results were obtained by Unsoy \textit{et al}. in chitosan nanocarrier because of the osmotic swelling property of this carrier at low pHs. Beside this ability of chitosan, ionic interactions with BTZ is high at low pH, so they choose potassium phosphate buffer at pH 6.0 as an optimum medium for obtaining best loading efficiency by providing a balance between osmotic and ionic characteristics of chitosan.\textsuperscript{7} Sensitivity to pH is also an important factor in BTZ release in tumor microenvironment with acidic pH. Researchers declared that in an equilibrium dialysis method BTZ showed 12 hours slow release (less than 5%) from dendrimer at pH 7.4 and 6 hours fast release (almost 57%) at pH 5, which were demonstrated good stability of this nanoparticle at physiological condition and targeted release at tumor microenvironment.\textsuperscript{31}

**Magnetic nanoparticles**

Magnetic nanoparticles had been introduced as another targeted drug delivery system. They have the ability to produce an efficient and precise drug delivery with minimum side effects through applying an external magnetic field.\textsuperscript{43} Alvarez \textit{et al}.\textsuperscript{16} composed a magnetic carrying system from an aqueous solution of ferric and ferrous salts at a molar ratio of 1:2 through co-precipitation method. They produced hyperthermia by magnetic field near the targeted tissue. The results proved that cytotoxicity of BTZ would be improved because of

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**Table 2.** Interaction sites of BTZ and polymers with pH responsive characteristics.

<table>
<thead>
<tr>
<th>Nanocarrier</th>
<th>Interacted moiety of carrier</th>
<th>Interacted moiety of BTZ</th>
<th>Type of interaction</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)</td>
<td>Carbonyl</td>
<td>Boronic acid</td>
<td>Ionic interaction</td>
<td>20</td>
</tr>
<tr>
<td>Polydopamine</td>
<td>Catechol</td>
<td>Boronic acid</td>
<td>Ionic interaction</td>
<td>24</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Amine</td>
<td>Boronic acid</td>
<td>Ionic interaction</td>
<td>7</td>
</tr>
<tr>
<td>Catechol-conjugated dendrimers</td>
<td>Catechol</td>
<td>Boronic acid</td>
<td>Ionic and hydrophobic interactions</td>
<td>27,29</td>
</tr>
<tr>
<td>Catechol containing poly(ethylene glycol) micelles</td>
<td>Catechol</td>
<td>Boric acid</td>
<td>Ionic interaction</td>
<td>31</td>
</tr>
</tbody>
</table>
microtubule disruption and aggresome formation in cells through magnetic field hyperthermia.

**Conclusion**

Bortezomib as a selective proteasome inhibitor is used in different tumors. Some polymeric nanoparticles would be introduced as an appropriate carrier due to their specific chemical structure. Hydrophobic and ionic interactions between BTZ and these systems are responsible for its efficient loading, sustained and targeted release in tumor site. Some novel methods such as using multifunctional and pH-sensitive polymeric carriers may help to develop BTZ efficacy in cancer treatment strategies.

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**Conflict of Interest**

The authors declare they have no conflict of interest.

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