Functional PEG Macromers for Biomedical Applications

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Abstract: Biomedical technology combines medicine and technology to diagnose, replace damaged site or regenerate tissue, and delivery of bioactive agent in a temporally controlled manner. Modified and unmodified natural and synthetic polymeric biomaterialsare currently formed in various structural shapes and chemical ingredients to overcome challenges. The recent developments in engineered PEG based hydrogel materials shows a great attractive research area due to its relatively high biocompatibility. The synthetic acrylated PEG or PEG-diacrylate and –multiacrylate monomers are the main backbone of photoinitiated radical polymerization of acrylates and polyesters used in non-degradable and degradable biomaterials. The physicochemical properties also enable to reinforce natural polymers structural characteristics. Recently published different classes of materials comprised of acrylated PEG macromers are summarized in this review.

Keywords: Biomaterials, hydrogel, poly(ethylene glycol)-diacrylate, poly(ethylene glycol)-multiacrylate, photo polymerization.

1. INTRODUCTION

Light-induced polymerization or UV-radiation curing is an efficient method to obtain a highly cross-linked solid polymer network which is insoluble in organic solvents and resistant to heat and mechanical stresses. The solvent-free formulation and ultra fast polymerization at ambient temperatures allow to find many application areas such as coating industry, manufacture of adhesives and composite materials, and microelectronics. Major UV-curable resins are acrylates or unsaturated polyesters, multifunctional epoxides and vinyl ethers. Polymerization mechanism initiates either by initiating species either homolytic photocleavage of aromatic ketones or by photolysis of arylonium salts with formation of protonic salts [1].

Poly(ethylene glycol)-diacrylate (PEGDA) macromers are diacrylated form of PEG (Figure **1**) and used in many potential biomedical application such as soft and bone tissue engineering scaffolds [2], controlled drug delivery [3], heart valve replacements [4] (Figure **1**). Hydrogel networks have formed with higher acrylate arms incorporated to PEG backbone. Recently, various hydrogel system where acrylated PEG have function either as structural backbone or adjuvant material. UV curing process is a highly preferred hydrogel formation method where photoinitiator and macromer/monomer formulation is the main essential point of network characteristics.

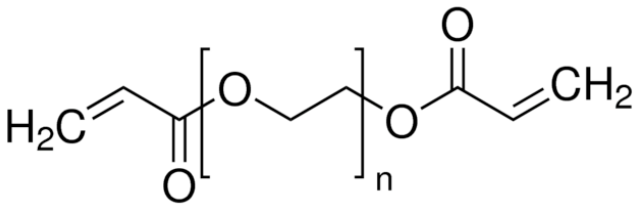

Figure 1: Chemical structure of PEGDA.

Hydrogel network systems based on natural or synthetic polymers have attractive research areas due to their vast processing parameters and chemical constituents. Cross-linked swollen polymeric building materials are frequently synthesized with degradable or nondegradable polymers based on the intended application [5]. Various methods were developed to form physical or chemical cross-linking of natural or synthetic polymers. The amount of free or bound water content determines the diffusion of less or high hydrophilic solutes through hydrogel. The inner hydrogel network characteristics like average pore size, pore size distribution and interconnections of pores needs to be analysed since these properties are greatly determined by the crosslinking density and chemical composition of the crosslinker used [6].

In this minireview the focus will be on chemically modified PEG which provide functionality in the synthesis of engineered tissue replacements, controlled drug delivery vesicles, cross-linker, and other designed biomedical related hydrogels.

2. TISSUE REGENERATION AND THERAPY

A scaffold should provide temporary biofunctional support like adhesion, proliferation, cell phenotype,

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E-ISSN: 2311-8717/17

matrix production, and enzyme activity. So, in order to mimic the natural structure, it is replacing, synthetic polymers and/or biological materials were used. Many experimental physicochemical characterizations are required in developing polymeric scaffolds such as surface properties, pore size, microstructure and interconnectivity, mechanical competence, etc. Scaffold fabrication methods also effect structural conformations [7]. The similarity of engineered tissue to targeted ECM gives the accomplishments of the process applied. The various forms of poly(ethylene glycol)-diacrylate based scaffolds, fibers, *Vs.* were fabricated for diverse application areas.

The ECMs are organized networks, which contain cell mixture of tissue or organs. This provides a physical scaffold besides cellular interaction processes of multi-molecular structures. Various approaches need to be developed in the case of interstitial or pericellular matrices. The abnormalities in ECM and cells are the crucial sings of the diseases that cause structural and compositional defects. The design of ECMs shows a huge investigations coming from the diverse biological activities and properties of the ECM components. The architecture, cyto-and tissue biocompatibility, bioactivity, and mechanical properties of ECMs are the fundamentals of tissue-engineered networks to mimic natural structures [8, 9].

A new type of biosynthetic hybrid scaffold was formed from the denatured fibrinogen fragments PEGylated with PEGDAs using photoinitiator and UV light curing process in the presence of a cell suspension. The hydrogel material provides mechanical properties for 3Dcell cultures while the continuity of biological functionality is performed by the backbone of the polymeric network. The molecular weight of the PEG constituents and polymer composition effected the elastic modulus of the scaffold while the biological domains in the fibrinogen backbone provide attachment motifs for smooth muscle cell adhesion in addition to proteolytic sensitivity for biodegradation. As a new generation three-dimensional tissue engineering scaffolds, the PEG-fibrinogen material supported endothelial cell stable adhesions, processes, and cellular extensions within the 3D designed structure [10]. In the fabrication of a multimaterial 3-D construct, two photocrosslinkable polymers were used as the primary scaffold materials. Multimaterial structures were manufactured by the addition of controlled concentrations of fluorescently labelled dextran, and bioactive PEG contained the tetra peptide Arg–Gly–Asp–Ser, and two photocroslinkable material, PEGDMA and PEGDA, with 1000 and 3400 Da molecular weights, respectively. The presence of the physically trapped or covalently attached fluorescent component to hydrogel was confirmed with fluorescent microscopy in the specificregions of the scaffolds fabricated with stereolithography down to 500 μm. The specific localization of fibroblast cells in in the regions patterned with bioactive PEG of multimaterial scaffold were analysed with phase contrast microscopy images [11]. Solvent-induced phase separation method was used to prepare inorganic–organic microporous hydrogel scaffolds based on methacrylated PDMSstar-MA) and PEGDA macromers. Enhanced modulus and degradation rates were obtained by a more even distribution of PDMS star-MA. The morphology, mechanical properties, swelling ratio, non-specific protein adhesion, bioactivity, controlled introduction of cell adhesion, and cytocompatibility of the hydrogels were characterized and offered as a useful materialto study guided cell behaviour and ultimate tissue regeneration [12].

Chitosan has a hydrophilic surface that promotes cell adhesion and proliferation due to its biocompatible, biodegradable and antimicrobial properties for use in tissue engineering scaffolds. But the use of the chitosan as the sole material for building scaffolds is rare due to its comparatively low mechanical strength. A research was conducted by incorporation of chitosan with a degree of deacetylation of 95% into a visible-light curable resin which is compatible with a digital light processing (DLP™) projection additive additive-manufacturing (3D printing) system. The research was continued with the production of a new scaffold where chitosan was added to a PCL-DA/PEGDA baseline resin. Different concentrations of chitosan were added to the photocurable resin and manufactured multi-layered scaffolds were evaluated for structure wettability, cell adhesion, and cell proliferation for future use in tissue engineering. Processing of biomaterial was not significantly altered by adding chitosan to PCL-DA/PEGDA resin [13].

Michael-type addition of amino compounds as a nucleophile to α,β-unsaturated carbonyl compounds is a well-known process. The N-alkylated photopolymerizable chitosan derivative (PEGDA-CS) was prepared under mild reaction conditions by Michael reaction between chitosan and PEGDA. The solubility of chitosan derivative demonstrated better results as water soluble derivative candidate and it could polymerize under ultraviolet light with a water soluble photoinitiator. PEGDA-CS showed antimicrobial

activity on *Escherichia coli* based on antimicrobial test results which application may expand in the fields of biomaterials by photo polymerization [14]. The biocompatible chitosan/PEGDA blend films were successfully prepared by Michael addition reaction in acidic solution. The weight ratios of films were changed from 100/0 to 0/100 in 10% increments. Structural, mechanical, and swelling analysis were performed to characterize the films. The potential wound dressing materials were nontoxic to mouse fibroblasts (L929) as reference cell lines [15]. An alternative tissue engineered vascular graft to autograft was produced by electro spinning technique. The coating of previously manufactured electrospun PCL fibrous mats was performed with different formulations of PEGA and PEGDA macromers, and photocrosslinkable GelMA. The macromers solutions photocroslinked in the presence of photoinitator under UV irradiation. The coated scaffolds were evaluated for both chemical/physical properties and interactions with blood and endothelial cells. The hydrogel coatings incorporating the two photo crosslinked PEG macromers showed the highest improvement of the hydrophilicity and wettability of the PCL mats. Endothelial cell adhesion and proliferation at the surface of the PCL, PCL-GelMA-10 (10% (w/v total solid content) and PCL-GelMA-1 (1% (w/v total solid content) materials were found to be high during the time frame of the study [16]. The spherical hydrogel microparticles that size and stiffness were independently tuned were synthesized using suspension photo polymerization for use as soft injectable tissue filler. Injectablemicro particles through 22 and 25 gauge needles were prepared from acrylated PEG, hyaluronic acid, gelatin and acrylated versions of hyaluronic acid and gelatin. Stirring speed, surfactant concentration, gelation time, photoinitiator concentration and UV intensity on microparticles size and yield were evaluated as process parameters and their effects on particle characteristics were revealed. Semi-IPN microparticles of PEG-DA and PEG were found cytocompatible by *in vitro* studies and PEG microparticles surfaces were functionalized with accessible amine groups at sufficient concentrations for potential surface modification [17]. Different photo-chemistry reactions, photoinitiators and ultra-violet (UV) exposure times were evaluated their contributions to undesired protein damage and cell death. PEGDA (3.4kDa) solutions were altered from 5 to 20% ww/v ratios and different stiffness magnitudes were measured. Thiolated proteins conjugated *via* thiol-ene to both acrylate and thiol-ene PEG hydrogel surfaces in order to produce bioactivehydrogel patterns. Bioactive hydrogels were created with vascular endothelial growth factor and ephrinB2 demonstrating its impact on endothelial cell behavior. Hence, bioactive hydrogels showed potential in extensive biological applications [18]. Tissue engineering substrates were formed *via* photo polymerization of PEGDA. The various molecular weights of monomer, initiator concentrations, and hydration rates were used to analyze their effects on mechanical properties of fabricated PEGDA hydrogels. An atomic force microscopy (AFM) based nano indentation method was used to measure the dry hydrogel samples prepared from three different PEGDA molecular weights (MW: 258, 575 and 700). The topography of hydrogel samples were imaged in non-contact mode and nano identitation was investigated by recording force and displacement of a three-dimentional indenter tip pressed into the surface. The polymerization process parameters mentioned above were observed to affect the mechanical behavior of PEGDA hydrogels from which the completely hydrated polymer showed mechanical properties similar to articular cartilage [19]. The valve replacements in current clinical options have the flexure limitation which represents a major mode of deformation directs there searches to create tissue engineered heart valves. PEGDA hydrogel scaffolds were produced and three-point bending tests were applied and the flexural stiffness was calculated. Molecular weights and weight fractions of PEGDA macromer in hydrogel synthesis were designed at three levels for each parameter. Hydrogels containing encapsulated valve cells, methacrylated heparin, or both reduced substantially stiffness compared with a cellular hydrogels. In conclusion, authors say that PEGDA hydrogels are an attractive potential scaffold system for tissue engineered heart valves because they are cytocompatible and modifiable but can also withstand bending deformations [20]. Photoactive PEGDA hydrogel surfaces were used to create patterned surfaces which have different feature sizes and shapes by transparency-based photolithography. In order to investigate the effects of UV irradiation time and precursor solution concentration on surface patterning feature sizes were determined. Human dermal fibroblast adhesion onto acryloyl–PEG–cell adhesion peptide patterned hydrogels were investigated [21]. Wide and deep 3-D channels which have six different wide and deep dimensions patterned into PEG hydrogels were reproducibly fabricated using a thiol-ene photo polymerized mold. Skeletal muscle cells were seeded and cultured in sterile PEG scaffolds. Notably, good cell alignment relative to the channel walls was achieved in relatively large channel

dimensions, for the case only when cells were cultured in multiple layers. All channel dimensions investigated promoted my oblast differentiation and multinucleated my otubes [22]. In a research, PEG based hydrogels were fabricated through sequential photo polymerization in the form of multilayers on the surfaces derivatized with eosin. The photo-polymerization technique allows the preparation of hydrogels under mild conditions using light to make the method attractive for various biomedical applications like tissue engineering, drug delivery, biomaterials, etc. The sequential formation of a crosslinked PEG hydrogel multi layers was applied on the initiator immobilized glass surfaces. The first layer was functionalized with eosin to form the second layer on top the first one. , The last layer may be formed using PEGDA alone or incorporating NH_2-PEG-acrylate precursor solution depending on the desired layers. The described approach can be used to form multifunctional 3-D hydrogel structures according to authors proposal [23]. A label-free non-invasive monitoring technique, a chemical exchange saturation transfer magnetic resonance imaging (CEST MRI) was selected to measure the dynamic changes in composite injectable hydrogel (Hyaluronic acid, Gelatin, and PEGDA, MW: 3400 g/mole) *in vivo*. CEST MR images of injected hydrogel into the brain of immunodeficient *rag2*$^{-/-}$micewere obtained at day 1 and 7 post-transplantation. *In vivo*, a significant decrease in CEST signal was observed at 1 week post-implantation which is consistent with the biodegradation of the gelatin component as validated by fluorescent microscopy of implanted hydrogels containing labeled gelatin[24]. PEGDA as the monomer and LAP as the initiator were combined to fabricate hydrogel scaffolds. A visible light-based projection stereo lithography (VL-PSL) system was developed to produce hydrogel scaffolds with desired shapes and internal architectures from computer aided design generated 3D models. Human adiposederivedstem cells (hADSCs) and Insulin-Transferr in-Selenium (ITS) were incorporated into scaffolds during the PSL process. Their fabrication of PEG hydrogels using VL-PSL results were found successful with designed geometries and internal architectures. The encapsulation of hADSCs retained high and long-term viability as live cell scaffold fabrication for *in vitro* tissue engineering and *in vivo* tissue repair [25]. A micrometer-sized bead that matches the dimensions of a typical cell, attached to the atomic force microscopy (AFM) cantilever in order to characterize the elasticity of hydrogel formulations intended to mimic physical properties *in vivo*. Agarose, alginate, the collagens, fibrin, hyaluronic acid, kerateine, laminin, Matrigel, polyacrylamide, PEGDA and siliconeelastomer (polydimethyl siloxane) were used to prepare various hydrogels. The elasticity of hydrogels at the micrometer-sized scale were measured by manipulation of the concentration of biomaterials. Additionally, force at the nanoNewton level, which is the force level relevant to single cells since the biomaterials tested is used widely for cell culture [26]. PEG-based hydrogels were generated from bis-cysteine matrix metalloproteinase-sensitive peptides and PEGDA (MW: 3400 kDa) macromers using step-growth polymerization. A second radical mediated photo polymerization step provided the cross linking process into hydrogel. The promotion of angiogenesis of the hydrogel system was tested by ex vivo aortic arch explants assay and endothelial cells from embryonic chick aortic arches embedded into hydrogels mediated the formation and invasion of new sprouts. The chemical procedure and significant cellular responses may aid to design or refine new materials for tissue engineering approaches [27]. The chemical crosslinking mechanism, conjugate thiol-ene Michael addition was selected to achieve rapid gelation under ambient conditions for an injectable PEG-based(PEGDA: Mw: 400 kDa) hydrogel system. Gelation between thiol and acrylate was realized in aqueous media and resulted with a hydrogel formation that has an elastic modulus of 189. 8kPa which is a mechanical property value similar to soft human tissue. The sustained release of methylprednisolone sodium succinate facilitated release over a concentration independent time with high encapsulation efficiency and programmable dosage. Hydrogels were functionalized by incorporating the oligolysinepeptide (Cys-(Lys)14-Cys) peptide on hydrogels to promote cell adhesion. The attachment of freshly isolated murine mesenchymal stem cells was reported for a definite value [28]. Polymerization mechanism and network structure of PEG hydrogels were investigated for photo polymerization of acrylates (chain-growth) or thiol-norbornenes (step-growth). Elevated intracellular reactive oxygen species and neo-tissue that resembles hypertrophic cartilage while intracellular reactive oxygen species content minimalized in the thio-norbornene system which resembles hyaline cartilage ECM [29]. Hyaluronan is a powerful tissue engineering tool since it is a major constituent of the extra cellular matrix (ECM), and the only non-sulphated glycosaminoglycan (GAG). Thus, HA and its derivatives are used in cell-containing hydrogels. Two thiolated hyaluronan (HA) derivatives and cysteine were coupled to PEGDA, PEG-dimethacrylate

(PEGDM), PEG-diacrylamide (PEGDAA) and PEG dimethacrylamide (PEGDMA). Human tracheal scar fibroblasts cell cultured in 3-thiopropanoyl hydrazide derivative (HA-DTPH) -PEGDA hydrogels *in vitro* showed high cell viability as an in situ crosslink able, injectable material for tissue engineering [30]. Two four armed PEG derivatives with different PEG chain lengths than converted to Tetra PEG tetra-acrylate derivatives (Tetra PAc). Acryl ate derivatized structures were used to couple with thiolated HA and gelatin derivatives into extrudable synthetic ECM hydrogels. The theological property, high shear storage modulus of cross-linked Tetra PAc was more suited to bio printing high-density cell suspensions. TetraPAc-crosslinked sECMs were equivalent or superior to PEGDA-crosslinked gels by encapsulation of NIH3T3 cells in printed tubular hydrogel macro filaments [31].

A new approach was proposed to fabricate controllable multiscale hierarchical structures using capillary force lithography, that can mimic topography and orientation cues of the ECM [32]. The single or multicellular morphology and orientation on multiscale biomimetic substrate or ECM was controlled by patterning the nanotopography with various orientations on the microgrooves *in vitro*. Capillary force lithography and original wrinkle technique were used to fabricate multiscale hierarchical structures. The patterned nanotopography with various orientations on the microgrooves and polyurethane acrylate (PUA) was applied without any deformation in the periodic wrinkles of a PEGDA mold on a UV/O-treated PDMS sheet. The fibronectin deposition on the patterned substrata was quantified higher than micro patterned structure for nanotopographic substrata when fibroblast cells were cultured [33]. The PEG hydrogel conjugated with a collagen mimetic peptide (CMP) of a specific, –(Pro–Hyp–Gly)$_x$–amino acid sequence with encapsulated chondrocytes was synthesized as a tissue engineering scaffold. The level of ECM (collagen and glycosaminoglycan, (GAG)) produced by chondrocytes was analyzed by GAG and total collagen assays in CMP-conjugated PEG gel. Results revealed that an 87% increase in glycosaminoglycan content and a 103% increase in collagen content compared to that of control PEG hydrogels occurred[34]. Two new types of PEGDA hydrogels were constructed by incorporation of differently charged monomers. The positive and negative charges were produced by incorporation of 2-(methacryloyloxy) ethyl-trimethylammonium chloride (MAETAC) and sodium methacrylate (SMA), respectively. The change in swelling ratio, cross linking density, and contact angel values were not observed between charged and non-charged hydrogels. Surface modification increased protein adsorption, irrespective of the charge polarity besides accelerated osteoblast-like cell attachment and raised the expression of cell attachment-related genes [35]. In a research, the conventional 3D printing and freeze-drying techniques were combined to produce lattice-type backbone and embedding microporous structures due to some limitations in three dimensional printing techniques. PEGDA and gelatin were used for the formation of the lattice-type backbone and microporous structures, respectively. The neural progenitor cells (NPCs) infiltrated and cell attachment; proliferation and differentiation were found to be satisfactory [36].

3. MULTIFUNCTIONAL PEG ACRYLATE MACROMERS

In addition to diacrylated PEG, more than two acrylate groups containing chemical structures, namely PEG-multiacrylate materials were extensively used in recent years.

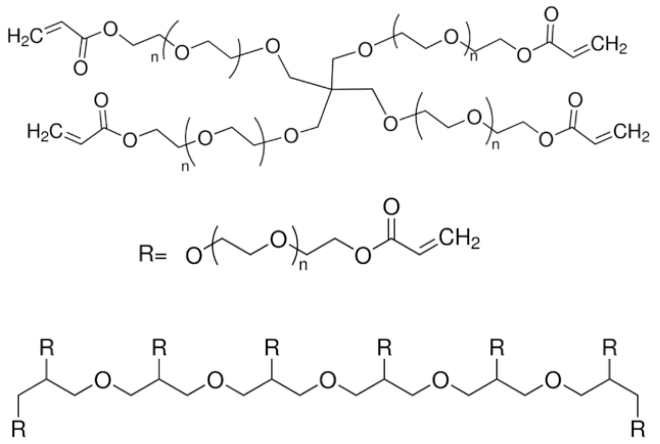

Figure 2: The structures of monofunctional; (**a**) 4-arm PEG 10K acrylate, (**b**) 8-arm PEG 10K acrylates.

A protein-polymer mosaic structure was created as a biological ECM analog material from fibrinogen conjugated with PEGDA where 4-arm star PEGMA (Figure **2a**) were added as cross-linker[37]. The controlled albumin release from tetraacrylate and octaacrylate PEG gels resulted with different release profiles[38]. The starting components of hydrogels were adjusted so that the release of encapsulated protein ranged from zero-order to sustained release over months. The hydrogel networks was conjugated by Michael-type reaction of an eight-arm PEG acrylate (Figure **2b**) dithiothreitol (DTT) [39]. Drug delivery devices based on PEGMA and acrylic acid were synthesized by solvent-free photo polymerization

method. The polymer network structure and the polymer properties were shown as design parameters to control the release characteristics [40].

4. BIODEGRADABLE COMPOSITES

The *in vivo* biodegradation of biomaterials may occur through a wide variety of mechanisms such as enzymatic or cellular breakage. On the other hand, since the mechanisms are also biomaterial dependent processes, biomaterial synthesis needs to be considered related to the device and its end-use application. The natural and / or synthetic biodegradable polymeric biomaterials including di- or multi-acrylated PEG were prepared for different medical purposes.

In vitro degradation characteristics of PEGDA and PEGDAA hydrogels were investigated under accelerated hydrolytic and oxidative conditions. The hydrolysis of acrylate esters of PEGDA was reported as *in vivo* degradation mechanisms while PEGDAA maintained its hydrogel properties which may be selected for long-term applications of hydrogels[41]. The influence of nutrient conduit network on the tensile strength for various photoinitiator concentrations, temperatures and incubation times of human DP147 mesenchymal fibroblasts cells were compared with the no networked PEGDA scaffold. The high photoinitiator concentrations and no networked hydrogels did not supported cell viability[42]. The effects of PEGylated protein adjuvants, albumin, fibrinogen, and gelatin, on the of subcutaneously implanted PEGDA hydrogel degradations were characterized by the use of magnetic resonance imaging (MRI)-based non-invasive manner. The role of protein constituents, including albumin, fibrinogen and gelatin in the *in vivo* resorption of PEG-based hydrogels implant was characterized by magnetic resonance imaging (MRI) by incorporating a Gd(AA)3-based contrast agent into acrylate-functionalized polymeric hydrogel biomaterials. Hydrogels were designed to exhibit similar material properties, including modulus, swelling and hydrolytic degradation kinetics. It was demonstrated that biodegradation properties of the PEG-Fibrinogen, PEG-Gelatin, PEG-Albumin and PEGDA hydrogels implanted in a rat subcutaneous model varied when MR-imaging and immuno histochemistry data were analysed for up to 25 days at set time intervals. It was predicted that the degradation of various PEG-protein hydrogels occurred *via* different mechanism than PEG hydrogels and this difference attributed to a reduced foreign body response [43]. The degradable polymeric hydrogels were synthesized by polymerization of PEGDA (MW: 400), a primary diamine, TTD, and acrylate-functionalized curcumin (a polyphenolic antioxidant) based on a Michael-type addition. The hydrophilicity, crosslink density, and overall flexibility of the network structure were altered by curcumin content which affects the degradation and release profiles [44]. Acrylated PEGDA was used in drug delivery devices either alone or conjugated with other molecules through various chemical reactions. The personalized anti-acne drug loaded masks/patches produced by two different 3D printing techniques which have special shape and size adapted to individual patient. It was additionally reported that the thermal degradation of drug depends on the 3D printing method[45]. The dextran–allyl isocyanate–ethylamine (Dex-AE) and PEGDA biodegradable composite hydrogel was prepared through UV photo-cross linking method and albumin release was regulated by pH sensitivity of the bio degradable hydrogels [46]. PEGDA and calcium carbonate were added as reactive diluent and buffering agent in order to study the *in vitro* degradation of methacrylicanhydrides of MCPH and MSA over a certain period under physiological conditions [47]. The *in vitro* long-term delivery of two macromolecules, horseradish peroxides (HRP) and bovine serum albumin was realized from an injectable network composed of MSA, MCPH, and PEGDA where PEGDA was reported as the crucial material for integrity during degradation [48].

Gelatin was incorporated into a PLEOF hydrogel cross-linked with PEGDA. The mechanical strength and degradation properties of these interpenetrating polymer networks (IPN) were optimized to acquire hydrogels with desirable mechanical and biological properties for bone repair [49]. A hepatocyte culture system for hepatic tissue engineering were synthesized by UV-initiated thiol-ene coupling polymerization of thiolated heparin (Hep-SH) and PEGDA. The albumin secretion and urea production of cultured hepatocytes on double heparin gel layers sustained several weeks. Additionally, heparin showed an affirmative role in stabilizing hepatocyte growth factor by protecting from proteolytic degradation [50]. A hydrogel-fibrous hydrogel scaffold composed of PLLA fibers combined with PEGDA hydrogel to improve viscoelastic properties for anterior cruciate ligament replacement. The best chemical release and mechanical properties was found for composite hydrogel soaked in 10 % hydrogel and the growth of fibroblast cells was sufficient compared to control, tissue culture polystyrene. The degradation behaviors up to 28 days

differed for various hydrogel contents [51]. The polyphenolic antioxidants quartering and curcumin were functionalized to form multiacrylate quartering and curcumin. Then the PAβAE hydrogels were produced by a single step addition of acrylates (PEG400DA and antioxidant multiacrylates) and a primary diamine, TTD. The oxidative stress and cytotoxicity of PAβAE hydrogeldegradation products results lead to a conclusion that the controlled delivery of polyphenolic antioxidants can be used in various drug delivery and tissue engineering applications [52]. The hydrogels made of NVP and HEMA were synthesized by the use of two different molecular weight PβA Ecross linkers. The swelling and degradation rates of PEGDA cross liked hydrogels were lower than the hydrogels cross linked with PβAE [53]. The combined effect of paclitaxel and iron oxide in the treatment of cancerous tissue was evaluated by the delivery of anticancer agent and heat from the biodegradable PβAE-based hydrogel nanocomposite. An alternating magnetic field was applied throughout the degradation process which leads to controlled paclitaxel release *via* bulk degradation of the hydrogels [54]. PEGDA, DEGDA, and isobutyl amine were combined to PβAE prior polymerization. The hydrophobicity / hydrophilicity properties due to combination as single or double macromers showed linear and multiphase degradation profiles [55]. A co-monomer system developed to fabricate a hydrogel, one of which is hydrophobic was reported. The cyclic acetal based hydrogels were formed from an EHD and PEGDA. It was estimated that physical properties of the hydrogels depend on initiator and monomer concentrations, and monomer ratios [56]. The long-term survival of encapsulated human dermal fibroblast cells was supported in a semi-IPN network with an optimized composition of PEGDA/HA. The PEGDA macromers with different hydrolytic degradation susceptibility were prepared by the synthesis of PEGDA macromers containing variable alkyl spacers [57]. A hydrogel architecture, PEGDA-PEI was designed such that its elastic modulus is equivalent to bulk plastic materials, and its controllable degradation rate behaviour was achieved by non-equilibrium swelling of the hydrogel [58]. The four PEGDA hydrogel formulations with similar degradation rates but differing moduli and mesh sizes were investigated on scaffolds design for encapsulated vocal fold fibroblast (VFF) ECM production. The scaffold mesh size was found as the critical regulator of ECM synthesis rather than the initial scaffold elastic modulus [59]. The pancreatic β-cells were encapsulated in PEG hydrogel fabricated by step-growth thiol-ene click reaction and compared with chain-growth photo polymerized PEGDA hydrogels. The cyto compatibility of thiolene reactions, the survival, proliferation, and formation of β-cells spheroids in this thiol-ene hydrogel system were investigated for 3-D culture of cells after certain times. The thiol-ene click reactions recommended as attractive means to fabricate gel platforms for tissue regeneration applications [60]. PEG-based hydrogels were synthesized *via* the step-growth polymerization of three different bis-cysteine matrix metalloproteinase (MMP)-sensitive peptides and PEGDA followed by radical mediated photo polymerization step. The collagenase sensitivity of MMP-sensitive PEG–peptide hydrogels and swelling of high molecular weight hydrogels increased. Additionally, significant *ex vivo* cellular responses were observed from embedded embryonic chick aortic arches [61]. A tethered cell adhesion ligand, YRGDS was immobilized to biomimetic PEGDA hydrogel systems which contains single and multiple collagen sensitive peptides domains within crosslinkes. The mechanical properties, and degradation rates were quantified besides encapsulated soluble acidic fibroblast growth factor (FGF-1) enhanced *in vitro* 3-D fibroblast cell invasion that provide degradation design criteria for tissue regeneration scaffolds [62]. The long-term cell viability of mouse fibroblast cells was investigated by the biological activity assays of adhered and migrated cells to the inner pores of copolymer (AP) hydrogel scaffolds made of alginate and unsaturated linear polyester poly(propylene fumarate) (PPF). The series of hydrogels were subjected to cross linking process with PEGDA and vinyl comonomers, HEMA, MMA and and N N' methylene bisacrylamide (NMBA) in order to alter physical and mechanical hydrogel properties [63]. An embolization plug composed of a radio-opaque filler and a PLGA blend was developed as a degradable radiopaque water triggered shape memory material. The compositions of PLGA composites was changed while weight percent of PEGDA which was used as coating material was kept almost constant [64]. The model protein, type A and B gelatin (Gel) was used in the fabrication of PEGDA hydrogel scaffolds containing Gel-PEG-Cys biomacromolecules. The covalent attachment of PEGDA and Gel-PEG-Cys resulted with a decreased gelatin dissolution out of the matrix and collagenase effect on degradation [65]. The PEI-PEGDA gels were loaded in multiple micro pockets of the PEGDMA hydrogel patch. Additionally, the bovine serum albumin labeled with fluoresce in isothiocyanate (FITC-BSA) or VEGF165 encapsulated PLGA microparticles were embedded in the hydrogel structure in order to in order to limit burst effect and

also attain the sustained release from the PEGDMA hydrogel patch. The molar ratio between PEI and PEGDA was tuned to alter the degradation rate of PEI-PEGDA gel, which regulates drug release from the hydrogels [66]. The desired features within a tissue engineered heart valve are mainly anisotropy, cell adhesion and viability, valvular interstitial cells (VIC)) activation and production of ECM. The stripe-patterned PEGDA hydrogels were created using the photolithographic patterning process and incorporation of cell adhesive and collagenase-degradable bioactive peptides into polymer networks were achieved. The heart valve tissue engineered hydrogel system showed the basis for fabrication and investigation of valvular interstitial cells culture [67]. Smooth muscle cells aligned with in 3D patterned channels was created on a PCL scaffold. This alignment was provided by physically coated of PEGDA and gelatin on PCL surfaces. The biological functions of smooth muscle cells (SMC) were evaluated for patterned and no patterned PCL substrates. Cell culture medium components affected hydrogel degradation, which gives possible *in vivo* biodegradation insight [68]. The maleic chitosan, a chemical modified structure of chitosan and PEGDA containing aqueous solution were photo polymerized to fabricate biodegradable hybrid hydrogel networks. Also according to their previous studies, crosslinking densities are the parameters that change the swelling and mechanical properties. Various maleic chitosan and PEGDA feed ratios are other process parameters to tune the mentioned properties. Maleic chitosan-based hybrid biodegradable hydrogel system showed low cytotoxicity to bovine aortic endothelial cells [69].

5. CONTROLLED DRUG DELIVERY

The three-dimensional insoluble polymer networks formed by covalent or physical crosslinking are another important therapeutic intervention in a wide range of health troubles. Controlled drug delivery in the use of diverse biomedical applications can be arranged by tuning the physical and chemical properties of hydrogels [69]. The biocompatible macromer, PEGDA plays an important ingredient in the synthesis of drug delivery systems as an easy accessible and highly hydrophilic polymer.

N-carboxymethyl chitosan and dibenzaldehyde-terminated PEGDA hydrogels were prepared as drug delivery vesicles. Inject ability, *in vivo* gel formation, and self-healing performance of pH sensitive hydrogels were tested. The activity results of the released anticancer agent, doxorubicinagainsthuman hepato-cellular liver carcinoma cells demonstrated the hydrogels as a new potential pH responsive injectable drug delivery device [71]. A synthetic strategy, two sequential cross-linking pathways relaying on two different cross-linking chemistries was developed for fabricating hemicellulose-based full IPNs with an enhanced hydrogel modulus. The primary single network of AcGGM utilizing vinylene moieties introduced on to the AcGGM backbone crosslinked into single-network hydrogels. The second-network cross-linking was performed either through a free-radical polymerization pathway using AAmand MBA monomers or with a thiol-ene click reaction between thiolated AcGGM-SH and PEGDA. IPNs achieved through thiol-ene reactions between thiolated AcGGM and PEGDA had shear storage modulus values 35–40 times higher than the single-network reference hydrogels as o potential material for interpenetrating drug delivery applications [72]. The single administration of vascular endothelial growth factor (VEGF) was not sufficient in the stabilization of tissues. Another growth factor, Angiopoietin-1 (ANG-1) besides VEGF was added to a polyethylene glycol-fibrinogen hydrogel carrier. A pro-angiogenic benefit from the sustained VEGF and ANG1 delivery highly increased restorative effect following acute myocardial infarction [73]. A photo-crosslinked PEGDA hydrogel reservoir containing the bi-specific protein stromal cell-derived factor 1 (SDF1) and glycoprotein VI (SDF1-GPVI) was generated as a drug releasing hydrogel. The functionality of SDF1-GPVI bi-specific protein after photo-crosslinking and controlled release *in vitro* leaded to offer the drug delivery system a powerful tool for therapeutic protein delivery in the treatment of cardiovascular ischemic disease [74]. PEGDA and HEMA hydrogels were cross-linked under mild photo initiating conditions at 30% and 50% w/w and 40% and 60% w/w ratios, respectively. The release and antimicrobial activity of incorporated gentamicin sulphate were tested. Drug release rates were found to be higher for low macromer contents in hydrogel formulations. Inhibition zone formations against *Pseudomonas aeruginosa* and *Staphylococcus aureus* microorganisms confirm that gentamicin sulphate preserves its antimicrobial activity after subjected to photo polymerization conditions [75]. Photo polymer-ized PEGDA composite hydrogels were fabricated by changing the molecular weights and ratios of gelatin. The concentration and molecular weight of natural polymer in the polymer network changed model drug gentamicin release behaviours compared with only PEGDA hydrogel behaviour [76]. The semi crystalline

PCL macromer was photocured with PEGDA to mechanically reinforce PEG hydrogels and induce shape memory behaviour. The swelling ratio and elastic modulus of hydrogels arranged by tuning the added PCL ratio. Silver loaded hydrogels exhibited antibacterial behaviour when inoculated using gram positive *S. aureus* and gram negative *P. Aeruginosa* strains for the case when used as drug delivery vesicle [77]. A Michael-type acceptor and donor were combined that gels in minutes to form a hydrophobic solid. The constant and partition-controlled release of progesterone were exhibited long term release from the cylinders having definite dimensions. The selected PEGDA macromers have 575 g/mol or 540 g/mol molecular weights. The partition-controlled release of progesterone has been achieved in an injectable waterborne, in situ-gelling material which may be a potential long-term, zero-order release system used to enhance the efficacy of the intrafallopian tube-gelling material for contraception [78]. The dosage effect of cell adhesion peptide Arg–Gly–Asp (YRGDS) incorporated into PEGDA hydrogel on osteogenesis of marrow stromal cells was examined. The osteogenesis of bone marrow-derived marrow stromal cells was promoted by the addition of cell adhesion peptide where the highest concentration of 2.5 mM was found as the optimum value. The expression of bone-related markers, osteocalcin and alkaline phosphatase increased with the same trend as mentioned [79]. The PEGDA macromer, crosslinker, and photoinitiator weight ratios were affected the dynamic swelling behaviour and gentamicin release while non-Fickian diffusion mechanisms were calculated [80]. The controlled release of the non-steroidal anti-inflammatory, antipyretic and analgesic drug, ketoprofen from 30 % and 50 % PEGDA hydrogel networks was investigated *in vitro* for three biological pH values. The drug release profiles were found to obey the Higuchi and first order kinetic models where high macromer amount was a factor to diminish the release [81]. EDTA is known as a chelating agent, inhibitor of metal-ion dependent proteases and platelet anticoagulant. The presence of 0.1 M EDTA concentration in PEGDA hydrogel networks facilitated gentamic in release even at higher cross linker ratios [82].

6. CROSS-LINKING APPLICATIONS OF FUNCTIONAL PEGDA

The transplantable gels with three stiffness levels of cellular microenvironment that influence hepatocyte structure and function have been developed. The stiffness of HA cell culture substrate was controlled by the addition of different cross linker, PEGDA concentration ratios. An intermediate stiffness between 1200 and 4600 Pa for maintenance of long term primary hepatocyte adhesion, functional marker expression, and morphological characteristics, *in vitro*, was reported as optimal stiffness [83]. Nanogels and microgels obtained by the free-radical polymerization of hyperbranched polyglycerol decaacrylate and PEGDA as macro cross-linker. Monodisperse microgels with uniform diameters of several tens or hundreds of micrometers were succeeded to produce since microgel diameters bigger than 50 mm is necessary to encapsulate cells. The viability of encapsulated yeast cells was estimated approximately as 30%, which was obtained by green staining [84]. The synthetic polymer PETis used in many biomedical devices. But inflammatory responses like leukocyte adhesion and fibrous encapsulation were elicited by this material. The thin films of pNIPAm-co-acrylic acidhydrogel microparticles (microgels) cross-linked with PEGDA were grafted onto PET surfaces using simple spin coating and cross-linking methods. The microgel coating of PET surfaces significantly reduced the fibrinogen adsorption and primary human monocyte/macrophage adhesion and spreading *in vitro.* The implantation of modified material in the murine intraperitoneal space again resulted with reduced leukocyte adhesion and expression of pro-inflammatory cytokines [85]. The content of cross-linker which is PEGDA with molecular weight Mn200 g/mol was changed in the fabrication of hydrogels from tannic acid, and polyacrylamide (PAAm) by semi-IPN and cryogelation techniques. The degradation rate of hydrogel with the lowest cross-linking density was estimated as the fastest rate with a remaining weight of approximately 13% after 70 days [86]. The hydrogel stiffness or cross-linker density was arranged using PEGDA as crosslinker in the synthesis of gelatin hydrogels which have polymerizable double bonds (methacrylic anhydride) and osteoinductive alendronate (Aln) grafted onto the gelatin backbone through aldehyde-activated reaction. The cross-linker and Aln density significantly improved the differentiation of bone mesenchymal stem cells analysed by the osteogenesis markers such as ALP activity, collagen type I and osteocalcin expression, and calcium deposition analysis methods [87]. The preparation of DIM imprinted material was performed in the presence of a monomer, allylamine and seven various cross-linkers. The binding capacity and selectivity of this material that participates in the suppression of viability of human ovarian and human breast cancer cell lines tested by

changing the cross-linkertype and ratio. In the research, six polar diacrylates or dimethacrylates of different lengths between double bonds, and one aromatic-divinylbenzene were compared while no selectivity towards the template was found when diacrylates added [88]. It was reported that the electro-optical performance of liquid crystal (LC)-polymer composites is important for obtaining better electro-optical performance. Prepolymer composition were adjusted byblending the PEG-diCi, EHA, and PFA at different weight ratios be sides aphotoinitiator and crosslinker PEGDA. An exact and clear micro-wall structure in preparation of flexible display devices was obtained due to phase separation between LC and fluorinated acrylate coming from solubility parameter difference [89].

7. MISCELLANEOUS APPLICATIONS OF PEGDA HYDROGELS

PEG hydrogel microstructures inside micro channels were fabricated using PEGDA having MW 575 as the base macromer by photolithography as bioreactor or biosensor. The alkaline phosphatase and urease containing micro reactors were designed as micro total analysis system and enzymatic catalysis of substrate was detected by change in emission intensity due to decreased pH in microchannels [90]. The synthesis of SMS-co-PEGDA micro sphere beads were achieved *via* suspension polymerization and the effect of amolecular weight PEGDA on a preparation of SMS-co-PEGDA microspheres and their Co(II) sorption behaviour were investigated. The roughness of the bead surfaces and particle size increased as the amount of SMS and the PEGD Aaverage molecular weights, Mn increased [91]. A colorimetric biosensor was constructed on a miniaturized platform by taking the benefits of the widely known unique characteristics of PDA (colour transition from blue to red by external stimuli such as temperature, pH, and other mechanical stresses). The reaction of PDA-embedded PEGDA hydrogel-based colorimetric biosensor demonstrated successful colorimetric analysis with different α-cyclodextrin concentrations as a model sample despite slow response [92]. In situ deactivation enhanced atom transfer radical co-polymerization of PEGDA, PEGMEMA, and $MEO_2MA$was applied to prepare a water-soluble hyperbranced structures [93]. A bi-layer chitosan/PAA/PEGDA composite membranes were prepared by UV-curing of PEGDA and then UV irradiation of chitosan to form nano layer on top of the previously formed hydrogel structure was performed [94]. The non-spherical chitosan—PEG microparticles were fabricated *via* the replica molding technique which offers simple, inexpensive and scalable production advantages. Single-stranded DNAs conjugated with Cu-free click chemistry method on micro particle surfaces can be used for bimolecular targets and applications based on the hybridization results with tobacco mosaic virus (TMV) *via* nucleic acid hybridization [95]. Glucose oxidase was encapsulated in PEGDA/gelatin composite hydrogels varying PEGDA ratio and gelatin molecular weights. The high PEGDA and gelatin ratios prevented the functionality of immobilized enzymes and relative enzyme activities were almost half of the low PEGDA ratio and low molecular weight gelatin containing hydrogels [96].

8. CONCLUDING REMARKS

Thus, this review aims to summarize the knowledge of recently reported researches that use PEGDA and PEGMA polymers, and its composites.

The chemical modification of PEG by the addition of acrylate groups enriched its utilization in biomedical hydrogels greatly due to allowing the formation of network without organic phase and hard conditions. Currently, the applications of hydrogel networks comprising acrylate functionalized PEG macromer especially with high molecular weights were mostly developed in the performing of *in vitro* and *in vivo* cell culture tissue engineering applications. PEGDA macromer also come into prominence in natural polymer-based biodegradable hydrogel structures either as crosslinker or mechanical strength adjuster. In the future, it is clear that the design of biomaterials with high biocompatible synthetic structures will proceed to be the most attractive research area to mimic natural constitution.

CONFLICT OF INTEREST STATEMENT

The author declares that there are no conflicts of interest.

ABBREVIATIONS

Poly(ethylene glycol)-diacrylate,

PEGDA: Poly(ethylene glycol)-multiacrylate.

PEGMA: Extracellular matrix.

ECM: Poly(ethylene glycol) dimethacrylate.

PEGDMA: Star poly dimethylsiloxane.

PDMSstar-MA: Poly (ε-caprolactone)-diacrylate.

PCL-DA: Gelatin methacrylamide.

GelMA: Poly(ethylene glycol) acrylate.

(PEGA): PEG diacrylamide.

PEGDAA: Lithium phenyl-2,4, 6-trimethylbenzoyl-phosphinate.

LAP: 4,7,10-trioxa-1, 13-tridecanediamine.

TTD: Sebacic acid dimethacrylate.

MSA: 1,6-bis-carboxyphenoxyhexanedimethacrylate.

MCPH: Poly(lactic-ethylene oxide fumarate.

PLEOF: Poly L-lactic acid.

PLLA: Poly(antioxidantβ-amino ester).

PAβAE: N-vinylpyrrolidone.

(NVP): 2-hydroxy ethyl methacrylate.

HEMA: Poly(β-amino esters).

PBAEs: Diethylene glycol diacrylate.

DEGDA: 5-ethyl-5-(hydroxymethyl)-b,b-dimethyl-1,3-dioxane-2-ethanol diacrylate.

EHD: Hyaluronic acid.

HA: Poly (ethylene imine).

PEI: Polyester poly(propylene fumarate).

PPF: Methyl methacrylate.

MMA: N N' methylene bis acrylamide.

NMBA: Poly (DL-lactide-co-glycolide).

PLGA: O-acetyl-galactoglucomannan.

AcGGM: Acrylamide.

AAm: N-N'-methylenebisacrylamide.

MBA: Poly(N-isopropylacrylamide).

pNIPAm: Poly(ethylene terephthalate).

PET: Polyacrylamide.

PAAm: 3,3′-diindolylmethane.

DIM: Polyethylene glycol-dicinnamate.

PEG-diCi: Ethylhexyl acrylate.

EHA: Pentafluorpropyl acrylate.

PFA: Sodium methallyl sulfonate.

SMS: Polydiacetylene.

PDA: Poly(ethylene glycol) methyl ether methacrylate.

PEGMEMA: (2-methoxyethoxy) ethyl methacrylate.

MEO2MA: Polyacrylic acid.

PAA: Ethylenediaminetetraacetic acid, EDTA.

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Received on 03-10-2017 Accepted on 29-10-2017 Published on 31-12-2017

DOI: https://doi.org/10.12974/2311-8717.2017.05.02.3