Review

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Biodegradable macromers for implant bulk and surface engineering

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Abstract: Macromers, polymeric molecules with at least two functional groups for cross-polymerization, are interesting materials to tailor mechanical, biochemical and degradative bulk and surface properties of implants for tissue regeneration. In this review we focus on macromers with at least one biodegradable building block. Manifold design options, such as choice of polymeric block(s), optional core molecule and reactive groups, as well as cross-co-polymerization with suitable anchor or linker molecules, allow the adaptation of macromer-based biomaterials towards specific application requirements in both hard and soft tissue regeneration. Implants can be manufactured from macromers using additive manufacturing as well as molding and templating approaches. This review

summarizes and discusses the overall concept of biodegradable macromers and recent approaches for macromer processing into implants as well as techniques for surface modification directed towards bone regeneration. These aspects are reviewed including a focus on the authors' contributions to the field through research within the collaborative research project Transregio 67.

Keywords: biodegradable polymeric blocks; implant surface modification; porous scaffolds; regenerative medicine; tissue engineering.

Introduction

Tissue defects exceeding a critical size require surgical implantation of a graft to ensure regeneration and restoration of functionality (Dinçel 2018). Especially autografts are typically limited by availability and co-morbidities (Pinho et al. 2016), while allo- and xenografts are compromised by the risk of disease transmission, immunogenicity and rejection (Safa and Buncke 2016). Artificial implants made from biomaterials represent an alternative that can overcome these restrictions (Sharma et al. 2019).

The term 'biomaterial' describes any material of natural or synthetic origin that is employed in contact with living cells and tissues (Vert et al. 2012). This definition includes metals, ceramics, glasses and polymers of both natural and synthetic origin (Baino et al. 2018; O'Brien 2011; Wilson 2018). Implants from polymers can be realized as solid monoliths, porous tissue engineering scaffolds or hydrogels, while metals, ceramics and glasses are typically used as macroporous scaffolds and as micro- and nanoparticles in polymer-based composites (Koons et al. 2020).

Biocompatible but otherwise inert biomaterials (first generation) and bioactive biomaterials (second generation) were still limited in the biological outcome of long-term regenerative applications (Hench and Polak 2002). With the emergence of tissue engineering as a biomedical field, the dependency of the regenerative process on cellular response to the implant became clear (Tozzi et al. 2016). The optimal outcome of a regenerative process is the *de novo* formation

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of tissue that is not distinguishable from its natural state. To this end, optimized biodegradability of the biomaterial became another key factor (Ogueri et al. 2019).

Both bulk and surface properties of an implant influence the cellular response and implant integration into the surrounding tissue. Bulk properties include mechanical properties of the material as well as implant shape, dimensions, porosity, pore size, and degradability (Hench and Thompson 2010). Mechanical properties of a biomaterial affect cell adhesion and differentiation (Friedemann et al. 2017), whereas pore sizes determine cell growth, metabolite transport and vascularization of the developing tissue (Bružauskaitė et al. 2016). For degradable implants, rate of degradation is an important parameter, as mismatched degradation kinetics and tissue regeneration rates are detrimental to the outcome of regeneration. In case degradation is faster than regeneration, implants could fail before the defect tissue is stable enough (Abdulghani and Mitchell 2019). An unfittingly slow degradation could inhibit continuous tissue formation as artificial material might still be present when new cell differentiation into new tissue has passed the proliferative phase (Dewey and Harley 2021). Alignment and directional orientation of a material may direct tissue growth both when implemented on the level of the biomaterial bulk as well as at the surface level (Lopresti et al. 2020; Omidinia-Anarkoli et al. 2017). Other surface properties include surface hydrophilicity and surface charge, interaction sites for specific attachment of cells, interaction with growth factor or cytokine receptors or the ability to release or deplete specific factors into or from the surrounding extracellular fluid. High surface hydrophilicity reduces unspecific cell attachment, while specific interactions of surface-bound attachment motifs and receptor ligands control growth and differentiation (Amani et al. 2019). Growth factors and cytokines can be incorporated into the biomaterial and released in a time- or enzyme-dependent mechanism (Bayer et al. 2015). Conversely, depleting the microenvironment of specific factors by scavenging via components of the biomaterial also influences cellular response (Lohmann et al. 2017).

Thus, in order to direct the regeneration process towards an optimal outcome, modern – third generation – biomaterials need to be adaptable with regard to both their bulk and their surface properties.

Macromers

Polymeric low molecular weight macromers composed of multiple building blocks with distinct functions are

perfectly suitable for the generation of biomaterials that can be adjusted in all these properties. Based on their composition, macromers need to be distinguished from macromonomers and telechelic polymers (telechelics). The IUPAC-defined term macromonomer describes polymer molecules with a single reactive group per molecule whose polymerization results in the generation of a polymer-grafted polymer (Nič et al. 2009). Telechelics are linear polymers or oligomers that are terminated with the same (homotelechelic) or different (heterotelechelic) reactive functional groups at either end. These reactive groups enable telechelics to function in further polymerizations or other reactions (Tasdelen et al. 2011; Vinciguerra et al. 2018). The term macromer, however, is used by us and others to describe macromolecules with at least one oligomeric or polymeric building block and at least two reactive groups per molecule, resulting in the generation of cross-linked materials (Hacker and Nawaz 2015; van Bochove and Grijpma 2019). In the most basic realization of such a macromer structure, a single oligo- or polymeric building block is terminated with a reactive group on both ends, yielding a homotelechelic such as poly(ethylene glycol) (PEG)-diacrylate (Figure 1(A)) (Cruise et al. 1998). In order to vary and adapt the properties of the resulting material, more complex macromers are designed with different building blocks and multi-armed core molecules to incorporate a higher number of reactive groups, as for example realized in macromers with polyester building blocks for the fabrication of a biodegradable hybrid silicate glass (Figure 1(B)) (Kascholke et al. 2017a) or in 8-armed, enzymatically cleavable macromers that can be cross-linked into hydrogels (Hesse et al. 2018). An even higher number of reactive groups and potential cross-linking sites can be achieved by integrating the reactive groups into the polymeric building block, as for instance in poly(propylene fumarate) (Figure 1(C)) (Kasper et al. 2009).

Types of polymeric building block

A wide variety of polymer types has been employed as building blocks of macromers. Typically, macromers for the use in implants employ degradable building blocks comprised of heterochain polymers. The most common degradable bonds are ester bonds and amide bonds. The categorization of polymers that contain esters as a functional group in the main chain is derived from the acid that is linked to a hydroxyl functionality (e.g. polyesters, polycarbonates, polyphosphoesters) (Hacker et al. 2019).

In a stricter sense, the term polyesters summarizes polymers containing ester-linkages between an organic

Figure 1: Schematic and exemplary chemical structures of macromers.

(A) Simple structure with a single oligomeric building block and two terminal reactive groups. (B) Complex structure with four arms and two different oligomeric building blocks per arm. Each arm is terminated by a reactive group. (C) Macromer in which the reactive groups are part of the oligomeric building block.

carboxyl functionality and a hydroxyl group. Polyester building blocks for macromers are generally reasonably hydrophobic, depending on the length, structure and stereochemistry of the carbon chain between the two functional groups (Spicer 2020). Their monomer structure can be comprised either of two alternating moieties, a diol and a dicarboxylic acid, or hydroxy acid monomers containing both a hydroxyl and carboxyl functionality (Hacker et al. 2019). Poly(butylene adipate) (Figure 2(A), $n = 2$ and $m = 2$) (Zhao et al. 2014) and poly(propylene fumarate) (Mott et al. 2016) are two examples of polyesters with alternating monomers that have been used as macromer building blocks. The introduction of a triol (i.e. glycerol) or tricarboxylic acid (i.e. citric acid) yields intrinsically branched structures that can be activated to obtain network-forming macromers (Gyawali et al. 2010; Singh et al. 2018). Common hydroxy acid polyester blocks in macromers are based on poly(lactic acid) (PLA) and its variants depending on monomer stereochemistry and tacticity (Figure 2(B), $R = -CH_3$) (Helminen et al. 2003; Jansen et al. 2011; Loth et al. 2015), its co-polymer with glycolic acid, poly(lactic acid-co-glycolic acid) (PLGA), with different ratios between the comonomers (Wilts et al. 2020) and poly(ϵ -caprolactone)

(Figure 2(C)) (Elomaa et al. 2011). All these polymeric blocks are easily accessible by ring-opening polymerization (ROP) of their cyclic mono- or diester precursors (Gentile et al. 2014; Kaihara et al. 2007). Due to the strongly decreasing solubility of poly(glycolic acid) (Figure 2(B), $R = -H$) blocks with increasing molecular weight, pure poly(glycolic acid) blocks have only recently been successfully incorporated into macromers (Guo et al. 2019).

Polycarbonates (Figure 2(D)) and polyphosphoesters (Figure 2(E)) are two additional types of biodegradable polymers based on esters of diols with inorganic carbonic or phosphoric acid, respectively (Bauer et al. 2017; Fukushima 2016). Both of them can serve as building blocks for macromers by ROP of cyclic monomers like trimethylene carbonate and ethyl ethylenephosphate (Steinbach and Wurm 2015; Weisgrab et al. 2020). Phosphoric acid can form three ester bonds per molecule, enabling modification of polyphosphoesters through the choice of the pendant ester during synthesis of the precursor monomers (Becker and Wurm 2018; Riva et al. 2020).

Modified monomers can further be used to alter the properties of the polymeric building block and the resulting macromers or to introduce additional sites for interaction

Polymeric building blocks

Reactive groups

Core molecules

Figure 2: Examples for building blocks of macromers.

Biodegradable oligo- or polymeric block: (A) Poly(di-ol dicarboxylate); (B) poly(α -hydroxy acid), $R = -CH_3$ poly(lactic acid), $R = -H$ poly(glycolic acid); (C) poly(ϵ -caprolactone); (D) poly(trimethylene carbonate); (E) poly(ethyl ethylenephosphate). Reactive groups: (F) acrylate ($R = -H$) and methacrylate ($R = -CH_3$); (G) vinyl ester/urethane; (H) maleic anhydride; (I) N-hydroxysuccinimide; (J) glycidyl ester/ether; (K) 3-(triethoxysilyl) propyl urethane. Core molecules: (K) 1,6-hexanediol; (L) trimethylolpropane; (M) pentaerythritol. The progression of a polymeric chain is indicated by *.

and/or modification. For example, PLA has been modified with amino acids through co-polymerization of a cyclic dimer of L-alanine and L-lactic acid (Kivijärvi et al. 2020), with azide functionalities through co-polymerization with azidomethyl-substituted trimethylene carbonate (Qiu et al. 2020), or with alkyl or aryl side chains through polymerization of substituted lactides (Trimaille et al. 2004).

In addition to biodegradable polymers, blocks consisting of non-degradable heterochain polymers can be commonly found in macromers as well. Oligo- or polyether blocks of ethylene glycol, for instance, are used to increase hydrophilicity of macromers (Hacker and Nawaz 2015). PEG blocks are often used for macromers for the fabrication of hydrogel scaffolds as they are biologically and chemically inert (Spicer 2020).

Reactive groups in macromers

In addition to oligo- or polymeric blocks, macromers are characterized by the presence of two or more reactive groups per macromolecule (Hacker and Nawaz 2015). Reactive groups in macromers refers to functional groups that result

in the formation of covalently cross-linked networks. Depending on the chemical cross-linking mechanism, these reactive groups can be distinguished into three types.

The first type comprises reactive groups that function as monomers in a polymerization reaction. Within this group, unsaturated double bonds are the most common example. Acrylates (Figure 2(F), $R = -H$) (Rose et al. 2017), methacrylates (Figure 2(F), $R = -CH_3$) (Levenhagen and Dadmun 2019) and vinyl carbonates or urethanes (Figure 2(G)) (Husár and Liska 2012) have been used to terminate macromer arms. Fumaric acid can either be used as monoalkyl ester for termination (Jansen et al. 2012) or, like its stereoisomer maleic acid, be integrated into the polymeric building block (Dhaliwal 2019; Gyawali et al. 2013; Mott et al. 2016).

The second type of reactive group includes functional groups that form covalent bonds through the reaction with complementary functional groups. Networks resulting from macromers with these functional groups need to be comprised of at least two components, with the second component being either a second type of macromer or a different molecule containing at least two of the complementary reactive groups. Reactive functional groups like

vinyls (Figure 2(G)), allyls, maleimides or norbornene can react with thiols in a thiol-ene addition (Fiedler et al. 2016; Hoff et al. 2020; Husár and Liska 2012; Northrop et al. 2015). Anhydrides and active esters like maleic anhydride (Figure 2(H)) or *N*-hydroxysuccinimide esters (Figure 2(I)) react with amines to form amides (Das and Theato 2016; Kascholke et al. 2017b). Epoxides like glycidyl (Figure 2(J)) can react with thiols, amines and other functional groups (Ekenseair et al. 2012; Muzammil et al. 2017). Thiols and amines are present on naturally occurring proteins, peptides and polysaccharides or can be synthetically added to a complementary macromer type. Other reactive groups like azides and alkynes for Huisgen cycloaddition or dienes and dienophiles for Diels-Alder and inverse electron demand Diels-Alder reactions are only accessible synthetically and need to be introduced during macromer synthesis or modification of naturally occurring macromolecules (Jiang et al. 2014).

The third type of reactive group describes functional groups that integrate themselves into a surrounding material by covalent or ionic interaction under formation of a hybrid composite. Examples include triethoxysilyl functionalities (Figure 2(K)) that integrate a macromer into a hybrid silicate glass (Kuzmenka et al. 2020) and free carboxyl functionalities from oligo(glutamate) blocks, poly(acrylic acid) blocks or hydrolyzed anhydrides that incorporate a macromer into calcium phosphate minerals (Bu et al. 2020; Simeonov et al. 2016).

Core molecules

Simple macromers consist of a linear sequence of one or more polymeric building blocks that are terminated by a reactive group at either end. Optionally, macromers can also contain a core molecule with at least two functional groups that serves as the initiator during synthesis of the polymeric building blocks. A core molecule with two compatible functional groups, such as 1,6-hexanediol (Figure 2(L)), results in linear macromers (Melchels et al. 2009). A core molecule with a higher number of suitable functional groups allows for the generation of branched macromers with multiple arms of the same structure. Trimethylolpropane (Figure 2(M)) is a three-armed core that has been used with a number of different ester-based polymeric building blocks and reactive groups for its low melting point, good miscibility with molten polymer precursors and distinct nuclear magnetic resonance (NMR) signal that allows for easy quantification of the molar mass of the polymeric building blocks per macromer (Loth et al. 2015; van Bochove and Seppala 2020). Other core

molecules used in literature are glycerol or pentaerythritol (Figure 2(N)) and their multimers as well as sorbitol and *meso*-erythritol (Brown et al. 2020; Kascholke et al. 2017a; Le Fer et al. 2019; Melchels et al. 2009; van Bochove et al. 2019). In the case of linear macromers with a central PEG block, the PEG block serves as core and initiator for additional, often biodegradable, polymeric blocks (Guo et al. 2019).

The TriLA material platform

The TriLA biodegradable materials that were developed as part of the Transregio 67 project are an example of macromers with adaptable compositions. The TriLA materials compose a platform of macromers with trimethylolpropane as the three-armed core, with each arm consisting of a degradable polymeric block, an optional oligo(ethylene glycol) block and methacrylate termination (Figure 3(A)) (Loth et al. 2015). For the biodegradable polyester block, the relatively hydrophobic monomers *D,L*-lactic acid, *L*-lactic acid and ϵ -caprolactone were chosen, with 2, 4 or 6 lactic acid monomers or 1 to 4 6-hydroxyhexanoic acid monomers per arm (Figure 4). For the development of the macromer platform, two different oligo(ethylene glycol) block lengths (on average 0.27 ethylene glycol units (Tri170) or 2.4 units (Tri450) per arm) were used in addition to macromers with trimethylolpropane (Tri134) as the core molecule (Figure 3(B)). The combination of two different polymeric blocks (i.e. polyether and polyester) allowed for tuning of arm length and number of available ester sites for cleavage. Mechanical stability of three-dimensional structures generated from these macromers decreased with increasing length of the polymeric blocks. With compressive moduli of LA-containing macromers ranging from 35 MPa for the shortest polymeric blocks to about 9 MPa for the longest block, macroporous scaffolds from the macromer showed comparable mechanical properties to cancellous bone and flexible skin (Figure 4). Increasing the length of the oligo(ethylene glycol) block resulted in a more pronounced decrease in compressive strength and moduli that could be linked to an increase in macromer hydrophilicity (Loth et al. 2015; Müller et al. 2017). TriLA macromers were synthesized with a maximum of six lactic acid monomers per arm in order to keep the mechanical properties of scaffolds in range for implants for bone regeneration (Figure 4), but incorporation of a higher number of monomers per arm has previously been proven possible (Jansen et al. 2013).

Initial degradation results of TriLA-macromer-based scaffolds at 37 °C revealed a continuous mass loss over a

Figure 3: General structure of the platform of three-armed biodegradable macromers referred to as TriLA materials, (A) with or (B) without the oligo(ethylene glycol) building blocks.

Figure 4: Compressive moduli of macroporous scaffolds (all approximately 78% porosity) generated from three-armed macromers of the design depicted in Figure 3, varying in molecular weight of the initiator (Tri###) and chemistry of the biodegradable blocks. LA, oligomeric D,L-lactic acid; LLA, oligomeric L-lactic acid; CL, oligomerized ϵ -caprolactone with LA/LLA/CL# referring to the theoretical number of hydroxy acid monomers per arm. Values are compared to literature-reported elastic moduli of different hard and soft tissues (cartilage highlighted in red, flexible skin highlighted in green and cancellous bone highlighted in blue). Reprinted from Loth et al. (2015) with permission from Elsevier.

period of months (Figure 5(A)) (Loth et al. 2015), compared to the sudden mass loss over a very short time following a period of stable mass caused by autocatalytic degradation processes that is characteristic for long chain PLA (Sevim and Pan 2018). More comprehensive investigations of macromer degradation are currently in progress and not yet published.

Macromers for the generation of biodegradable hybrid silicate glass scaffolds were subsequently derived from the

TriLA material platform (Kascholke et al. 2017a). The mechanical properties of the resulting hybrid glass were dependent on the macromer composition in a manner similar to scaffolds made from the cross-linkable macromers (cp. Figure 4) – with increasing length of the polymeric polyether and polyester blocks, compressive strength and moduli decreased for scaffolds fabricated with otherwise comparable parameters. The degradation profiles of these hybrid glasses, similarly to cross-linked

Figure 5: Degradation behavior of three-armed, biodegradable macromers illustrated as remaining relative dry weight (dry weight of sample relative to initial dry weight) over time in degradation medium (PBS at 37 °C).

(A) Degradation study on solid monoliths and macroporous scaffolds of the TrLA macromer Tri170(LA4)3.75. (B) Degradation study of selected hybrid bioglass scaffolds with TriLA-derived macromers containing reactive silanes. TriXXXLAY-ZZ refers to the composition of the macromer and hybrid bioglass (XXX molecular weight of the ethoxylated TMP core, Y length of the biodegradable blocks, ZZ the relative content (in %) of macromer in the hybrid bioglass). Reprinted from Loth et al. (2015) and Kascholke et al. (2017a) with permission from Elsevier.

TriLA networks, displayed linear mass loss over a period of almost a year for a concentration of 20% macromer relative to silica sol (Figure 5(B)). With a higher macromer concentration of 40%, the degradation rate increased, reaching complete dissolution after 168 days. Mass loss rates ranged from 0.2 wt.-%/d to about 0.5 wt.-%/d and correlated with macromer contents in the hybrid glasses and weight ratio of the degradable polyester block to non-degradable polyether block. Such a correlation has not yet been described in literature for hydrolytically degradable hybrid glasses.

Implant generation from macromers

In order to generate a functional implant, macromers need to be fabricated into three-dimensional, solid or macroporous structures through cross-polymerization of their reactive groups. The specific reaction mechanism and the available modes of fabrication depend on the type of reactive group as categorized previously. For instance, thermal or light-based initiation is used for cross-linking of polymerizable groups, whereas the integration of silane-based reactive groups into a hybrid glass is mediated through hydrolysis of the pendant alkoxy functionalities

and condensation with the silicate structure (Hendrikx et al. 2017). While solid implants can be generated by reacting macromers in a mold of the desired shape, macroporous scaffolds with μ m-sized pores require pore generating techniques or additive manufacturing (Abbasi et al. 2020). Choice of pore generating technique influences scaffold bulk properties such as total porosity, pore size, pore interconnectivity and interconnect size and surface properties such as pore surface morphology (Babaie and Bhaduri 2018). Porogen templating approaches were established with a wide variety of porogen materials that are leached from the structure after macromer cross-polymerization. Recent developments for porogen templating of macromers include reaction-induced phase decomposition with a non-reactive diluent (Rose et al. 2017), temperature-induced phase separation and subsequent photo-cross-linking (Wu et al. 2018) and crystallization of a solvent component before cross-linking for microporosity generation (Geven et al. 2017). Methods that were compatible with the processing of polymerizable macromers include emulsion templating with alginate as additional porogen for simultaneous generation of macro- and microporosity (Owen et al. 2020) as well as use of photo-degradable polymers as porogens (Dou et al. 2017).

Templating with solid lipid particles that partially melt at elevated temperatures into a continuous lipid phase to generate a well interconnected pore network was previously

employed for high molecular weight linear PLGA (Hacker et al. 2007). This lipid templating technique was recently adapted for macroporous scaffold generation with the thermally cross-polymerizable TriLA macromer platform developed in TRR67 (Loth et al. 2015). The adaptation required tuning of the polymerization temperature, lipid content and composition as well as initiator and macromer concentration to match the polymerization kinetics with lipid melting. Macroporous scaffolds were successfully fabricated with overall volume porosities of up to 88%. Implanted in an *in vivo* model, TriLA scaffolds with 75% volume porosity and coated with collagen and sulfated glycosaminoglycans increased bone formation (Picke et al. 2016). In order to further improve the osteogenic potential of TriLA scaffolds, fabrication and porogen parameters were optimized to yield scaffolds with increased pore sizes (average 210 μm) and comparable porosities (Krieghoff et al. 2019). The optimized scaffolds improved *in vitro* osteogenic differentiation and mineralization both directly through the wider pore size and indirectly through increased collagen and glycosaminoglycan immobilization. Previously, pore sizes exceeding 300 μm were generally reported necessary for improved vascularization that is conducive to bone regeneration (Karageorgiou and Kaplan 2005). However, pore sizes of 125–250 μm were recently shown to be adequate for improved vascularization if the pore interconnects were of sufficient diameter (here $55.7 \pm 14.4 \mu\text{m}$) (Gupte et al. 2018).

Templating approaches for macroporous scaffold are flexible and easy to set up, but yield random pore structures between replicates and restrict pore shape to the shape of the porogen. Additive Manufacturing (AM) techniques, also referred to as 3D-printing or rapid prototyping, work by realization of a computer-generated structure in a reproducible manner (Ngo et al. 2018). A wide variety of macromers with different polymeric blocks and reactive groups that can be polymerized by photo-initiation have been developed for generation of scaffolds and implants with light-based AM techniques like stereolithography or continuous digital light processing (Kuhnt et al. 2019; van Bochove and Grijpma 2019; van Bochove and Seppala 2020).

For macromers that cannot be directly processed through additive manufacturing, the advantages of reproducibility and freedom of construct design are achieved through indirect rapid prototyping. In this approach, negatives of the desired structure are additively manufactured from a soluble material, used as templates and subsequently removed from the final scaffold (Houben et al. 2017). Indirect rapid prototyping has been employed to generate constructs with porosities between 44 and 68%

and regular pore shape out of both non-degradable and degradable macromers for the formation of hybrid silicate glasses (Hendriks et al. 2016; Kascholke et al. 2017a).

In contrast to solid materials for skeletal defects, hydrogel implants with high water content are usually mechanically weaker and thus more interesting for softer tissues like skin or nerves (Madhusudanan et al. 2020). Bulk and surface properties of macromer-based hydrogels can be adapted by macromer composition, and macromers are also used for specific hydrogel functionalization, for instance with small amines to improve cellular attachment (Kohn et al. 2016). A single type or several different types of macromer are cross-linked by their reactive groups into a stable hydrogel (Smithmyer et al. 2019). Macromers can also be processed into porous hydrogels, for example in templating oligo(poly(ethylene glycol) fumarate) macromers with dried gelatin microspheres that are subsequently leached at elevated temperature (Wang et al. 2015). Macromers are also used to covalently cross-link a non-macromer hydrogel former into a multi-component material, for instance anhydride-containing macromers with gelatinous peptides (Kohn-Polster et al. 2017), thiol-containing macromers with poly(*N*-isopropylacrylamide-co-glycidyl methacrylate) (Guo et al. 2019) or cysteine-containing peptide-terminated star-PEG with maleimide-modified heparin (Spiller et al. 2019).

Scaffolds and surfaces

The surface of an implant represents the contact interface with cells and tissues (Mas-Moruno 2018). Surface characterization, analysis and specific design are therefore of great importance to a successful application *in vivo*. Interactions at the biomaterial surface typically depend on two main properties, namely surface topology and surface chemistry. Beyond the material's inherent bulk properties, a directed surface functionalization offers a versatile toolbox towards an improved material integration and thereby long-term impact on tissue regeneration (Bacakova et al. 2011; Rahmany and van Dyke 2013).

2D systems for characterization of macromer surfaces

While bulk properties, such as pore size and mechanical stability, can be conveniently determined for porous scaffolds (Krieghoff et al. 2019), a detailed analysis of the surface is often challenging. Since most methods for surface analysis are limited to flat surfaces, translation of the 3D

implant surface to 2D is required. To this end, a model system of cross-co-polymerization of TriLA macromers in order to project the properties of complex implant surfaces to films was established (Müller et al. 2017). This method yields 2D films that reflect the surface composition of previously established 3D structures made of the TriLA macromers (Loth et al. 2015). The methacrylate terminations of the TriLA macromers were found to be sufficiently reactive for cross-co-polymerization with other molecules. This enables a convenient and highly versatile approach for modifying the networks formed by the macromers (Gronbach et al. 2020a; Müller et al. 2017).

After cross-co-polymerization with methacrylated PEG linkers, TriLA films were successfully modified with alkaline phosphatase (ALP), a key enzyme in bone matrix mineralization, and with peptides containing the RGD motif for integrin-mediated cell adhesion (Müller et al. 2017). Subsequent analysis proved preservation of ALP activity as well as a significant increase in cell adhesion on RGD-functionalized films. Further attempts to functionalize the surface with glycosaminoglycans (GAGs) were limited by the relatively low density of PEG linker molecules on the surface, as a consequence of inhomogeneous distribution due to phase separation of hydrophilic PEG methacrylate from cross-polymerizing hydrophobic T134LA6. In order to address this problem, the functionalization concept was fundamentally reworked. Therefore, a sequential modification process was established (Figure 6) (Gronbach et al. 2020b). Instead of the methacrylated PEG-linker, the small anchor molecule glycidyl methacrylate (GMA) was incorporated into the macromer network during cross-co-polymerization. In a second, independent step, a polyetheramine linker (amino-terminated poly(ethylene glycol-co-propylene glycol)) was immobilized on the surface. This approach preserved the versatile adaptability of the underlying TriLA bulk material and in addition allowed for a high degree of freedom for

subsequent modification with various polyetheramine linkers in order to investigate the effect of reaction conditions, linker length and amount on the surface.

The impact of linker length and density on the presentation of bioactive molecules is extensively discussed in literature (Abstiens et al. 2019; Kapadia et al. 2019). Through the integrated amino group, a broad range of functionalization approaches is possible (Mas-Moruno 2018). It is therefore important to quantify the amino groups available on a biomaterial surface. To this end, TriLA films were incubated with an amine-reactive fluorescein derivate and the amount of covalently immobilized dye was quantified via high resolution fluorescence scanning (Gronbach et al. 2020b). Additionally, the immobilized amine-functionalized linker led to improved cell adhesion in comparison to PEG-modified surfaces (Gronbach et al. 2020a). In a comparable manner, amine-terminated PEG has been applied as a linker for subsequent functionalization (Stewart et al. 2019).

Surface functionalization strategies for bone regeneration

In the field of bone regeneration, surface functionalization of implants is a promising approach to guarantee an effective integration in the defect site (Figure 7). The applied strategies range from nanostructuring of surfaces to covalently bound bioactive macromolecules like antibodies or glycosaminoglycans (Förster et al. 2017; Stewart et al. 2019). Surface roughness and distinct surface patterns, as a type of physical functionalization, were shown to control cell-biomaterial interactions (Baptista et al. 2019). For example, nanostructured grooves (Bjørge et al. 2019) and dots (You et al. 2010) as well as periodical micropatterns (Lauria et al. 2016) were introduced to surfaces and synergistically improved the osteogenic differentiation of human mesenchymal stem cells (hMSC).

Figure 6: Macromer films are an accessible analytical tool for surface analysis.

The cross-co-polymerization of a small anchor (glycidyl methacrylate) and subsequent linker (polyetheramines) decoration offers highly versatile surface functionalization options, e.g. with highly sulfated hyaluronans (Gronbach et al. 2020a,b).

Figure 7: Surface functionalization approaches for 3D-scaffolds as implants.

1: Structured surface patterns (You et al. 2010), 2: Local release of active substances (Agrawal and Sinha 2017), 3: Coating with artificial extracellular matrices (aECM) (Krieghoff et al. 2019; Rother et al. 2015), 4: Improvement of cell adhesive properties via RGD peptide immobilization (Müller et al. 2017), 5: Presentation of immobilized growth factors (He et al. 2008), 6: Complex scavenging via GAG immobilization (Gronbach et al. 2020a), 7: Enzyme immobilization (Müller et al. 2017), 8: Antibody immobilization (Lima et al. 2018).

A common approach aims at the presentation of beneficial molecules to the material surroundings. Different methods can be applied for this kind of surface decoration. They range from physical adsorption, mediated by hydrophobic or electrostatic interactions as well as hydrogen bonds, to covalent immobilization of complex proteins. Molecules that are commonly immobilized via adsorption are structural proteins of the extracellular matrix, such as fibronectin (Heller et al. 2015), laminin (Rahmany and van Dyke 2013), vitronectin and collagen (Ao et al. 2014), all of which carry adhesion motifs that allow cells to bind to the modified surfaces through integrins (Barczyk et al. 2010). Glycosaminoglycans can be combined with adhesion proteins as they show affinity to defined protein domains on adhesion proteins, such as heparin-binding domains on fibronectin (Teixeira et al. 2020). Similarly, fibrillary collagen type I has been shown to bind sulfated hyaluronans. Coatings of this combination of extracellular matrix (ECM) components on TriLA scaffolds have been shown to support osteogenic differentiation (Krieghoff et al. 2019) and improve bone regeneration in a diabetic mouse model (Picke et al. 2016). Growth factors, such as the potent osteoinductive bone morphogenetic protein-2 (BMP-2) can be effectively adsorbed to material surfaces directly or after modification with extracellular matrix proteins (Agrawal and Sinha 2017). This process can be defined as sequestration, if the growth factor can be released via cell-demanded enzymatic ECM or

material degradation. Moreover, an interaction with the ECM via an allosteric binding site may provide enhanced bioactivity of the presented growth factor (Belair et al. 2014).

A general drawback of adsorptive surface modification with proteins is that their functionality strongly depends on the biomaterial properties (Lewandowska et al. 1992). Hence, bioactivity and availability for the surrounding cells and tissues following loading and storage are difficult to predict.

A more controlled surface presentation is provided by covalent immobilization of small bioactive molecules. This has been shown for small RGD peptides that are easier to handle than complex ECM-proteins. They have been bound to the surface of a linker-modified polymer or macromer network (Lieb et al. 2005; Müller et al. 2017), covalently linked to a macromer network directly (Shin et al. 2011), cross-co-polymerized during macromer network formation (Behravesh and Mikos 2003) or used in functionalized PEG-based macromers (Phelps et al. 2012). The spatial presentation of RGD peptides was shown to strongly influence cell behavior on the functionalized surfaces and should be taken into account (Bilem et al. 2018; Maheshwari et al. 2000). However, this approach has only been realized on silanized model surfaces, not with macromers.

More complex proteins, such as BMP-2, may also benefit from covalent immobilization, which for example has been shown on modified titanium implants (Mont et al.

2004; Teng et al. 2016) and in PEG-diacrylate macromer hydrogels (Masters 2011). However, care has to be taken to avoid inactivation by cross-linking or denaturation of the protein. In general, controlled grafting to a surface provides the opportunity for a precise design of the functionalization. This includes the generation of patterns by linker-guided covalent surface decoration (Bilem et al. 2018) or growth factor gradients which, in the case of BMP-2, allow for spatial control of osteogenic differentiation on material films (Crouzier et al. 2009; Lagunas et al. 2013). Stromal cell-derived factor 1 α (SDF-1 α) has been grafted via azide-alkyne cycloaddition to TriLA films and controlled release of anti-inflammatory SDF-1 α via a matrix metalloproteinase-9 (MMP-9)-sensitive peptidic linker was shown (Steinhagen et al. 2014). In addition to the immobilization of either growth factor or adhesion motive, the combination of fibronectin and BMP-2 (Brigaud et al. 2017; Ren et al. 2011) or RGD and BMP-2-derived peptides on biomaterial surfaces (Moore et al. 2011) synergistically enhanced osteogenic differentiation. An indirect approach applied for immobilization of BMP-2 involved covalent grafting of heparan sulfate for adsorptive immobilization onto a biodegradable polymer (Edlund et al. 2008).

In some approaches the necessary linker for functionalization adds beneficial functionalities to the material. An example is PEG, which is widely applied as a linker for biofunctionalization, e.g. with RGD peptides, and additionally introduces anti-fouling properties to the material (Buxadera-Palomero et al. 2017; Subbiahdoss et al. 2010). Nevertheless, an emerging number of studies report on the formation of anti-PEG antibodies in the patient, which may lead to considerable immune reactions (Yang and Lai 2015). This illustrates the need for modified or novel linker strategies, especially those that yield bioinert surfaces, i.e. water soluble polyphosphoesters (Pelosi et al. 2020).

Complementary to the release of osteoanabolic molecules from biomaterials or their local surface presentation via sequestering, beneficial interference via inactivation of potentially catabolic factors in the material surroundings is widely discussed in the field of biomaterials. Such a scavenging could result in a directed functional inactivation of e.g. catabolic molecules through strong interaction with the material. We and others define scavenging as functional inactivation of such bioactive molecules by virtually irreversible binding to a material (Lohmann et al. 2017). As a prominent example, antibodies have been immobilized to material surfaces, which then inactivate further signaling, e.g. via pro-inflammatory cytokines (Zhang et al. 2020). The functionalization of biomaterials with antibodies offers

high availability at the site of action. This avoids the dose-limiting side-effects that have to be taken into account when antibodies are applied systemically (Hansel et al. 2010). In addition, this approach enables a local use of antibodies in areas that could not be reached by freely circulating antibodies as well as an increased residence time due to their conjugation to the biomaterial (Washburn et al. 2013). One example for this approach is the neutralization of Interleukin-6 via antibody-functionalized polymeric particles made of hyaluronic acid and chitosan. The functionalized particles were designed for intra-articular injection and thereby a local treatment of arthritic diseases (Lima et al. 2018).

Next to antibodies, which comprise a well-defined, specific binding site, every other strong binding partner may potentially be used for local scavenging of a bioactive molecule. This has been realized in glycan-functionalized microgels that were found to specifically scavenge lectins and aim at inactivating bacterial toxins due to defined binding motifs (Jans et al. 2017). A comparable observation was made when GAG-containing macromer-based hydrogels were investigated in an impaired wound healing model in mice (Lohmann et al. 2017). The study showed effective scavenging of the inflammatory chemokines MCP-1, IL-8, and MIP-1 α by star-PEG-GAG gels which resulted in superior wound healing when the star-PEG-GAG gels were applied. For sulfated GAGs, a strong affinity towards the Wnt-antagonist sclerostin has been shown (Salbach-Hirsch et al. 2015). In studies with the TriLA film system, covalent functionalization with the highly sulfated glycosaminoglycan hyaluronan (sHA3) resulted in a directed scavenging of Wnt antagonists sclerostin and DKK1 (Gronbach et al. 2020a,b). In a competitive binding experiment both antagonists were found to be the preferred binding partners of sHA3 over the pro-osteogenic agonist Wnt3a (Figure 8). The concept of directed scavenging was successfully transferred to a cell culture environment. Consequently, the impact of the functionalization on the osteogenic differentiation of SaOS-2 cells and hMSC was shown. Cells seeded on sHA3-functionalized materials expressed significantly higher ALP activity and matrix mineralization in comparison to control groups. In summary, a sequential surface functionalization approach was successfully applied to modify TriLA-based material with sHA3. A pro-osteogenic effect of the successful scavenging of Wnt antagonists has been demonstrated *in vitro*. Ongoing studies focus on a transfer of these results to the surfaces of three-dimensional constructs, such as macroporous scaffolds or microparticles that could be administered by injection.

Figure 8: Impact of sHA3 functionalization on the amount of Wnt antagonists (DKK1, sclerostin) and Wnt3a in cell culture supernatant of hMSC and effect of directed scavenging on osteogenic differentiation (Gronbach et al. 2020b).

Applications of macromers beyond implant generation

Macromer applications in the previous sections mainly concerned implant generation for the treatment of *in vivo* tissue defects. Even more reports on macromer-based biomaterial design for *in vitro* applications in tissue engineering, regenerative medicine and drug delivery are available that illustrate the high adaptability of this material concept. In tissue engineering, macromers have been investigated to generate scaffolds for *ex vivo* cell culture in addition to *in vivo* implants, for instance for the expansion of stem cells from patients or the growth of specific tissue-like cell networks (Le et al. 2020; Murphy et al. 2020). For drug delivery, macromers are investigated for both covalent and non-covalent drug encapsulation in macro-sized implantable depots or for the generation of micro- and nanoparticles for injectable formulations (Haesslein et al. 2008; Jansen et al. 2011; Secret et al. 2014; Stillman et al. 2020). Degradable macromers which release the bound drug during the degradation process are especially attractive for application in which a steady and sustained drug release is desired (Elmowafy et al. 2019; Hacker et al. 2009; Ueda et al. 2007).

Conclusion

Structural alterations of biodegradable macromers, especially chemistry of building blocks, molecular weight of polymeric blocks and type and density of reactive groups, allow for a wide ranging adjustment of bulk and surface properties of cross-polymerized

networks. The chemical design options are further extended by cross-co-polymerization of macromers with heterobifunctional linker molecules that enable biological modifications via bioconjugation reactions in adjustable density. With regard to structural design, the comparably low molecular weights of macromers is the main reason for their good adaptability to different fabrication techniques including rapid prototyping which may lead to a variety of new biomaterials in application-specific as well as patient-specific design. With our TriLA macromers, we contribute to the body of biodegradable macromers that, for the listed reasons, hold promise as versatile platforms for implant generation, bioactive materials for tissue engineering, regenerative medicine and drug delivery.

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