Bioapplications of Networks Based on Photo-Cross-Linked Hyperbranched Polymers

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Summary: This article deals with some of the most recent developments in the use of hyperbranched polymers in biomedical applications. Some examples have been selected to show their potential in drug delivery, tissue engineering, imaging technologies and molecular imprinting. Moreover, the preparation of methacrylic networks using chemically-modified hyperbranched polymers as multifunctional macromonomers by photopolymerization is described. The capability to support valve interstitial cell culture was demonstrated and the adhesion and functionality of the cells was related to the mechanical properties of these materials.

Keywords: biological applications of polymers; biomaterials; hyperbranched polymers; macromonomers; networks; photopolymerization; tissue engineering

Introduction

UV-curing technology has been implemented for multifarious applications in sectors such as paints and coatings, adhesives, electronic devices, imaging, stereolithography and holography. Photopolymerization[1] is a versatile and advantageous technique to produce polymeric materials and, recently, also nanocomposites. For these reasons, its applications have been spread off to the biomedical arena.[2–4] Temporal and spatial control of photopolymerization endows a powerful methodology to produce ex- and in-vivo biomaterials. Recent developments are focused on biomimetic materials,[5] cell encapsulation,[6,7] tissue engineering,[8] drug delivery,[9] molecular imprinting[10] and microfluidic devices,[11] among others.

The performance of the material depends on the nature of the components of the photocurable formulation together with the interaction between them. The main ingredients in a photocurable formulation are: (i) the photoinitiator, that absorbs UV light and generates active species which are capable to induce the transformation from a viscous liquid to a solid material; (ii) the monomers of different nature that play the role of modifying the curing rate, the viscosity, the volatility, the toxicity and the physical properties of the final material and (iii) the cross-linking agents (multifunctional monomers or telechelic polymers with reactive chain ends) that provide structural stability by generating the three-dimensional network (and affecting also the nature and properties of the cross-linked material). There are other important components that have to be taken into account depending on the application. For example, macromolecular oligomers that decrease contraction during curing, and may increase the viscosity of the formulations facilitating their deposition.

One of the most promising areas of research concerns the improvement of macromolecular properties by controlling the macromolecular architecture. In the last decade, hyperbranched polymers (HBPs) have been incorporated in the UV-curing technology for preparing thermoset coatings,

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taking advantage of the high number of reactive end-groups and their particular topology.

HBPs are dendritic polymers characterized by a tree-like architecture which confers upon them very different properties compared to their linear counterparts. It is generally accepted that the globular end-functionalized structure gives rise to a lower degree of entanglement (lower viscosity) and an enhancement of the end-chain effects.[12] The structural control in HBPs through the adjustment of their degree of branching or by the modification of their end-groups allows the fine-tuning of their physical properties and optimizing them for their applications.[13] The potential of HBPs on radiation curing has been shown by a wide number of applications, some of them already commercially developed. Compared to dendrimers, the synthesis of HBPs is easier and more cost-effective. Although some scientists have pointed out that the poorly defined structure and the relatively high polydispersity of HBPs might restrict their applications, novel synthetic methods have recently been developed, in which substantially better-defined HBPs with narrow molecular distributions have been obtained.[14] For small-scale applications, mainly in the biomedical sector, the development of products based on dendrimers is an emerging field.[15–17] An alternative to dendrimers is to consider HBPs as building blocks to construct novel materials for treating human diseases.

Recent developments in novel polymer architectures have rendered other three-dimensional polymers such as covalent amphiphilic polymer conetworks. These materials may exhibit different morphologies enabling surface reorganization of hydrophobic and hydrophilic domains and diffusion of biomolecules. Conetworks have properties that make them suitable for biomedical applications including applications for drug delivery, contact lenses,[18] tissue engineering,[19] and storage and delivery of DNA.[20–21]

This article deals with some of the most recent developments in the use of HBPs in biomedical applications and describes briefly our results in preparing culture platforms for valvular interstitial cell (VIC) characterization in vitro using them as multifunctional macromonomers.

Biomedical Applications

HBPs have found applications in drug delivery,[22] tissue engineering, imaging technologies[23] and molecular imprinting. The advantages of HBPs for these applications are based on their size (working as nanocarriers) and their tunable peripheral-functionality, favoring interactions with drugs, genes, image contrast-agents, biomacromolecules and cells. Environment-sensitive HBPs, such as pH-, temperature-sensitive and salt-triggered polymers, have been prepared in order to be employed as stimulus-responsive systems, for example, in drug delivery.[24]

Polymer drug delivery technologies aim to deliver drugs effectively and efficiently and to improve the biopharmaceutical and pharmacokinetic properties of drugs.[25] Dendritic polymers have achieved high intracellular drug levels.[26] Moreover, they may have a high charge density with tunable surface functional groups, which can alter the local environment and influence cellular interactions such as the cell uptake mechanism and its subsequent intracellular transport which are also dependent on the type of cells targeted.[27] It is worthy to note the enhanced availability of the end-groups, which do not penetrate in the core of the macromolecular coil, compared to that of linear polymers. New glycerol-based hyperbranched polycarbonates were prepared and the presence of glycerol and carbonate residues in the repeating unit makes the polymer potentially biodegradable and biocompatible and, therefore, a promising material for drug delivery.[28] Low toxicity and biocompatibility of hyperbranched polyether polyols were the incentives to develop new functionalized derivatives based on these polymers that could be used as non-viral gene delivery systems.[29]

The transfection efficiency was comparable to that of polyethyleneimine, while limited cytotoxicity rendered these HBPs more attractive as gene carriers.

The chemical functionality of HBP end-groups can be modified to make molecules with novel biological properties that exploit polyvalent and cooperative receptor-ligand interactions.[30] HBPs have also been used as heme protein models.[31] New synthetic substitutes for human serum albumin, the most abundant plasma protein in the body, were prepared based on hyperbranched polyglycerol whose properties seem promising and closely mimic the compatibility, binding and transport properties of the natural material.[32]

HBPs and dendrimers offer interesting alternatives to generate synthetic macrotemplates for macromolecular imprinting.[33] Molecular imprinting involves the synthesis of polymers in the presence of a template to produce complementary binding sites with specific recognition ability. Several approaches have been explored during the development of biomacromolecule imprinting.[34,35] However, the objectives of templates with specific interactions and high selectivity are difficult to reach due to the natural complexity of biomacromolecules. For example, the conformational flexibility of proteins brings about non-specific binding and the high number of functional groups results in an increase of cross-reactions and decrease in selectivity. HBPs have been used as templates due to their ability to mimic the size, the shape and the functionalities of biomacromolecules. Also the synthetic strategy may be addressed to incorporate degradable bonds to make the removal of the template easier. Selleger and Hall have prepared molecularly imprinted polymers (MIP) using HBPs as templates, which have found application for phase-affinity separation or sensing of relevant proteins, polynucleotides or polysacccharides.[36] The use of HBPs as templates for MIPs is a promising approach as far as it extends the applications allowing the synthesis and analysis in a wider range of environments than those for biomacromolecules whose bioactivity is sensitive to operational conditions (pH, temperature, solvent).

Tissue engineering strategies involve a temporary biomaterial to provide mechanical support and cell adhesion, and biological factors are commonly incorporated to control the cell activity. Recently, one of the authors of this article has extensively reviewed the use of macromolecular monomers for the synthesis of hydrogel niches by photoinitiated polymerization and their applications in cell encapsulation and tissue engineering.[37] The current directions relate primarily to the requirements for facile approaches to modulate network structure and to incorporate diverse biological functionalities. The outstanding properties of HBPs make them promising materials to achieve these requirements. However, there are only a few articles describing the use of HBPs in tissue engineering.[38]

UV-Curable Scaffolds for Tissue Engineering

Cross-linker technology provides a crucial tool for developing materials for use in optical devices[39] and biomedical applications.[40] In this regard, hyperbranched macromonomers (HBMm) offer the possibility to control network cross-linking by modifying the number and nature of reactive end-groups.[41] Recently, we have modified HBPs with terminal methacryloyl groups to be used as cross-linkers for the preparation of biomaterials (Figure 1). The photoinitiated polymerization of several methacrylic monomers was examined in the presence of the HBMm and bis(2,4,6-trimethylbenzoyl)phenylphosphine oxide (Irgacure 819®) as a photoinitiator, upon UV irradiation.[42] Fully-cured networks were obtained by using HBMm as cross-linkers during the UV-curing of methacrylic monomers.

HBMms copolymerize with methacrylic monomers allowing one to overcome topological factors and vitrification that would limit final conversion of conventional

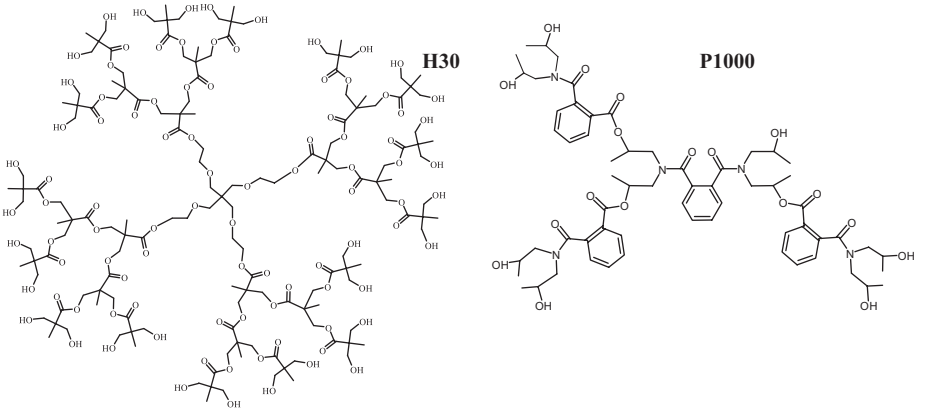

Figure 1.
Idealized structure of the hyperbranched polyester Boltorn® H30 and Hybrane® P1000. Modified macromonomers contain an average number of 8 methacrylic groups for H30MA and 5 for P1000MA.

multifunctional monomers. The incorporation of HBMm in UV-curable methacrylic monomers caused an increase of the free volume fraction as confirmed by fluorescence.[42] As HBMm concentration increased, the gelation point was delayed, and it, therefore, resulted in an increase of the final conversion. This behavior was dependent on the concentration and the structures of the monomers and HBMm. A good correspondence was found between the gelation point and the final conversion (α_{tgel} and α_F, respectively), as shown in Figure 2.

Morphology investigation by SEM and AFM showed phase separation leading to a dispersed particulate/hole structure by increasing the amount of HBMm. H-bonding and π-stacking might induce self-assembly of HBMm leading to reaction-induced phase separation and to materials with enhanced toughening characteristics. Moreover, the characteristics of the photo-cross-linked networks as scaffolds for tissue engineering applications were evaluated. Preliminary results have shown that hyperbranched cross-linked methacrylic networks supported VIC growth and adhesion,

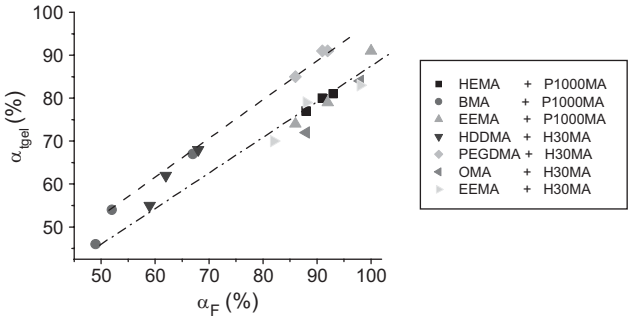

Figure 2.
Plot of α_{tgel} as a function of α_F for the UV-curing of several mono- and difunctional methacrylic monomers in the presence of different amounts of H30MA and P1000MA. HEMA: 2-hydroxyethyl methacrylate, BMA: butyl methacrylate, EHMA: 2-ethylhexyl methacrylate, HDDMA: 1,6-hexanediol methacrylate, OMA: octyl methacrylate, EEMA: ethoxyethyl methacrylate, PEGDMA: poly(ethylene glycol) dimethacrylate (PEGDMA, M_n 575).

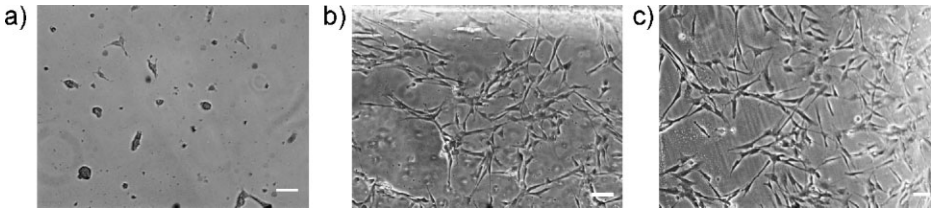

Figure 3.
Micrographs of VICs seeded into methacrylic materials based on 2-hydroxyethyl methacrylate (HEMA), without cross-linker (a), with 8 wt% HBPm cross-linker H30MA and with after 48 h (b) and 72 h (c). Scale bar, 50 μm.

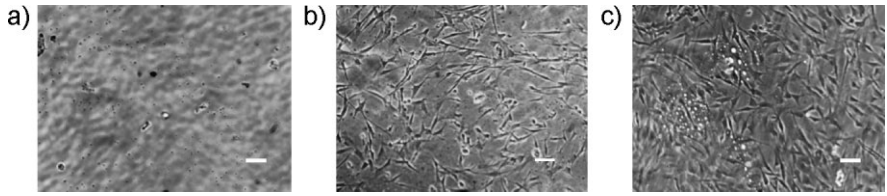

Figure 4.
Brightfield image of VICs seeded in networks based on poly(ethylene glycol) dimethacrylate (a) and hyperbranched macromonomers H30MA (b) and P1000MA (c). Scale bar, 50 μm.

with no cell toxicity (Figure 3). This result is attributed to the high conversion the methacrylic groups reached using HBMm as cross-linkers, reducing the presence of free monomers in the material and, therefore, its toxicity.[43] The activation of the VIC myofibroblast phenotype was also related to the presence and amount of HBMm. Induction of myofibroblast activation facilitates aortic valve remodeling but is also related to alterations in valve matrix architecture and, consequently, in fibrotic disease.[44]

Cell attachment was greater onto the networks photogenerated in the presence of the highest amount of HBMm. This behavior has been related to the stiffness and hydrophilicity of the substrate. It has been described previously that the presence of H30MA in HEMA based materials[42] leads to more rigid networks, while maintaining or enhancing the swelling ratio. These special properties are due to the reaction-induced phase separation that results in a porous/particulate structure. Phase separation can be also induced by a molecular self-assembly process of P1000MA, constructing a different morphology that may affect cell spreading (Figure 4). Cell adhesion and shape in hydrophilic PEG films can be tuned by substitution or by modifying HBMm concentration. In this regard, mechanical stiffness of substrate materials represents an additional parameter that can regulate adhesion of and subsequent colonization by viable bacteria[45] and cells.[46] Hyperbranched polymer H30MA, when copolymerized with HEMA, provokes a steep decline in hydrophilicity in relation to its concentration.

Conclusion

The cost-effective synthesis of polymers with highly branched architecture and exposing end-groups at their globular periphery has opened a wide range of opportunities to the preparation of novel materials for biomedical applications such as drug delivery, tissue engineering, imaging technologies and molecular imprinting.

The multiple surface groups can be selectively engineered, allowing the fine-tuning of their physical and chemical

properties. The modification of the number, the position and the nature directly influence the cross-linking density of the resulting networks and the mechanical properties.

The incorporation of HBMms to UV-curable methacrylic monomers causes an increase of the free volume fraction as confirmed by fluorescence measurements. As the HBMm concentration increases, the gelation point is delayed, resulting in an increase of the final conversion. This behavior is dependent on the concentration and the structure of the HBMm and the comonomers. HBMm exhibited a favorable role for the understanding and manipulation of VIC properties through the alteration of the stiffness of the substrate as well as the degree of methacrylic group final conversion. This study can be translated to the preparation of scaffolds for valve tissue regeneration.

Overall, the multifunctional HBP cross-linkers afforded networks with promising properties that suggest that these may be suitable for tissue engineering applications. Dendritic polymers will play a significant role in the future development of new biomedical applications for the treatment of human diseases.

Experimental Part

Photopolymerization

Methacrylic monomers were photopolymerized in bulk in the presence of different amounts of methacrylic end-capped hyperbranched polymers, HBMm. The photoinitiator (1%, w/w with respect to the total weight of monomers) and the fluorescent probe (0.002%, w/w) were added to the samples. Samples were irradiated under nitrogen with a Sylvania 400 W Hg-medium-pressure lamp. The simultaneous measuring of the fluorescence of the probe and the heat evolved in the polymerization was performed by coupling DSC with a spectrofluorometer. Hydrophilicity of the resulting films was calculated by contact angle measurements against water.

Cell culture

Valvular interstitial cells were isolated by sequential collagenase digestion of aortic valve leaflets VICs and cultured in growth media consisting of 15% fetal bovine serum (FBS), 2% penicillin/streptomycin, 0.2% gentamicin in Media 199 at 37 °C in a 5% CO_2 environment. VICs were seeded at a concentration of 50 000 cells cm^{-2}.

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