Photopolymerizable Elastomers for Vascular Tissue Regeneration

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Summary: The aim of this study was to design new resin formulations for blood vessel substitutes with small inner diameter that can be 3D-printed by Additive Manufacturing (AM). Commercially available urethane oligomer acrylates as cross-linking agents (CAs) with different reactive diluents (RDs) and/or thiol chain transfer agents (CTAs) were examined. It could be shown that the properties of photopolymers of carefully selected CA/RD/CTA combinations can be varied in a wide range, also to fit with those of natural blood vessels. Moreover, these materials showed good biocompatibility in in-vitro cell culture tests with endothelial cells. A new method to assess the tear resistance of the new materials in comparison with natural blood vessels was designed and established. The tear resistance of the developed photopolymers already approaches those of natural material, although there is still need of improvement. The 3D-structuring of optimized resin system succeeded. Hence AM has proven to be an ideal tool to manufacture parts with the complex structure of natural blood vessels.

Keywords: additive manufacturing; biocompatibility; biomaterial; elastomers; photopolymerization

Introduction

Life style diseases, especially diseases of the cardiovascular system, are one of the main causes of morbidity and mortality in the industrial countries. The coronary heart disease is caused by partial or total occlusion of a coronary artery. The treatment of this disease is typically an aorto-coronary bypass operation. Up to now autologous bypass materials (autografts) are state of the art. Materials from cadaveric human or animal donors (allografts or xenografts) are not suitable alternatives since the immune response cannot be suppressed sufficiently. As an alternative, artificial blood vessels (synthografts) can be used for vascular reconstruction. For example expanded polytetrafluorethylene (ePTFE) or polyethylene terephthalate (PET) are well established materials.[1] Unfortunately, these materials are only suitable for large diameter reconstructions since synthetic grafts with an inner diameter of less than 5 mm tend to lose patency due to thrombogenesis and inflammation. Biodegradable, polyester based materials like polyglycolic acid (PGA) or polylactic acid (PLA)[2] overcome this shortcoming of the traditional material as they promote the tissue regeneration while they are resorbed. However the main disadvantage of these materials is the high degradation velocity, the involved

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fast decreasing mechanical properties and the liberated high amount of acids that can cause inflammation reactions or in the worst case necrosis.

Additionally, complex cellular structures as desired for artificial grafts can hardly be manufactured out of these materials, because for biocompatibility and long-term patency of the grafts not only the chemical constitution but also the structure and mechanical properties of the materials play a decisive role.[3] Therefore, we are interested in the development of suitable blood vessel substitutes out of biocompatible, mechanically tailorable photopolymers. We designed our resin formulation to be suitable for rapid prototyping which offers us the high range of shaping possibilities of additive manufacturing (AM).[4]

Selection of the Material

In prior studies, different acrylate-based monomers were screened concerning their applicability in regenerative medicine.[5–7] However, most of these photopolymers turned out to be not suitable for artificial vascular graft materials due to their hard and brittle material behavior. An exception was cyanoethyl acrylate for it fulfills the basic material requirements for vascular tissue regeneration as photopolymerizable hydrogels with a PEG-based crosslinking agent (CA).[8] Unfortunately, there are still some drawbacks especially concerning the suture tear resistance. Therefore, new base monomers (Table 1) with different reactive diluents (RDs, Table 2) and chain transfer agents[9] (CTAs, Table 3) were screened. Urethane based resins were selected as base monomers because they are known to have good mechanical properties and biocompatibility. Monofuctional RDs with different side chains (urethane-, aliphatic-, hydroxy- and cyano-groups) were chosen to modify the properties of the base monomers. As CTAs, the well known tetrafunctional TT and the difunctional ether group containing DOD as potential flexibilizer were chosen. Improved mechanical properties can be expected due to step-growth mechanism and the high uniformity of crosslinking.[10]

A large variety of different combinations of base monomers, RDs and CTAs – were photopolymerized and rated concerning their flexibility, elasticity and strength. The photopolymerization of the specimens with a thickness of about 2 mm was performed in silicone moulds with a bisbenzoyl phosphine oxide-based photoinitiator by the aid of a UV-lamp system (1 kW dysprosium doped high pressure mercury-lamp, 25 cm distance, 5 min exposure in air).

Best results were obtained for photopolymers of formulations containing the difunctional base monomer H1, the RD HEA and the CTA DOD. For this reason, this combination was examined in a wider context of testing (Table 4 and Table 5).

Tensile Tests

The mechanical properties of natural blood vessels (ovine A. carotis) were tested for comparison using a tensile testing machine (Zwick Z050). The testing parameters were testing length $L_0 = 5$ mm and velocity $v_t = 5$ mm/min. The results for tensile modulus (E), elongation at break (ε) and tensile strength (S) were $E = 450(\pm 100)$kPa, $\varepsilon = 130(\pm 22)\%$ and $S = 1010(\pm 120)$ kPa.

To test the photopolymers, formulations with and without CTA (Table 4 and Table 5) were photopolymerized, shoul-

Table 1.
Base monomers.

Abbr.	Brand (Company)	Description	Mol. wt. [g/mol]
H1	Genomer 4215 (Rahn)	Aliphatic polyester urethane diacrylate	1500
H2	Genomer 4188/EHA (Rahn)	Urethane monacrylat in 20% EHA	-
H3	Photomer 6210 (Cognis)	Aliphatic urethane arcylate oligomer	1400

Table 2.
Reactive diluents.

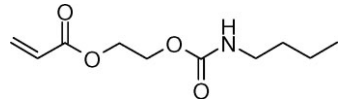
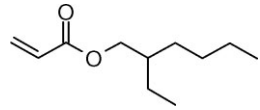
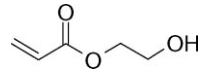
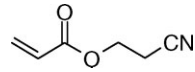
Abbr.	Substance	Structure	Mol. wt. [g/mol]
BEA	2-(((Butylamino)carbonyl)-oxy)ethyl acrylate		215.25
EHA	2-Ethylhexyl acrylate		184.28
HEA	2-Hydroxyethyl acrylate		116.12
CEA	2-Cyanoethyl acrylate		125.13

Table 3.
Chain tranfer agents.

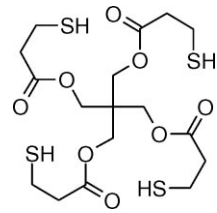
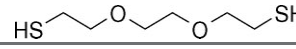
Abbr.	Substance	Structure	Mol. wt. [g/mol]
TT	Pentaerythrit tetrakis(3-mercaptopropionate)		488.66
DOD	3,6-Dioxa-1,8-octane dithiol		182.31

Table 4.
Formulations without CTA.

Formulation	I	II	III	IV	V
X[a)]	*5*	*15*	*20*	*25*	*30*
n (H1) [mmol]	37.58	20.07	16.27	13.69	11.81
n(HEA) [mmol]	375.8	602.0	651.0	684.4	708.6
wt% (H1)	56.4	30.1	24.4	20.5	17.7
wt% (HEA)	43.6	69.9	75.6	79.5	82.3

[a)]Molar ratio of double bonds in HEA:H1.

Table 5.
Formulations with DOD.

Formulation[b)]	VI	VII	VIII	IX
Y[c)]	*128*	*64*	*32*	*21*
n (H1) [mmol]	19.88	19.71	19.36	19.02
n(HEA) [mmol]	596.5	591.2	580.7	570.7
n(DOD) [mmol]	4.97	9.85	19.36	28.53
wt% (H1)	29.8	29.6	29.0	28.5
wt% (HEA)	69.3	68.6	67.4	66.3
wt% (DOD)	0.9	1.8	3.5	5.2

[b)]Formulation II with a ratio of functional groups in HEA to H1 of 15 was taken as basis.
[c)]Molar ratio of double bonds to thiol groups in the resin.

dered test bars (according to ISO 527) were punched and tested in tension (Figure 1).

As expected, the tensile strength as well as the modulus decreases and the ultimate strain increases with increasing ratio of RD. This is due to the reduced network density within the photoelastomers (Figure 2, left). Similar effects were observed for the photoelastomers containing CTA. However, in this case the decrease of strength and modulus and the increase of ultimate strain, respectively, can be attributed to the decreased chain length of the backbone of the photoelastomers (Figure 2 , right).

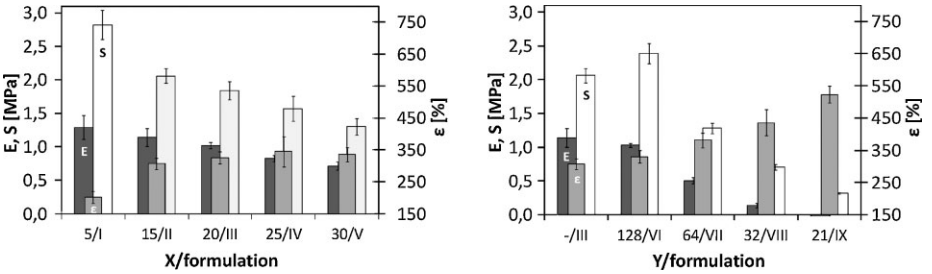


Figure 1.
Mechanical properties of the photopolymers without (left) and with (right) CTA.

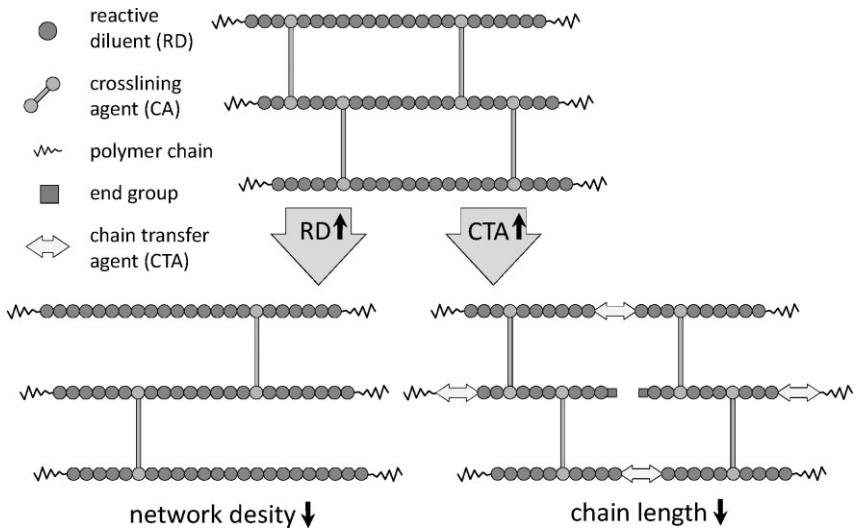


Figure 2.
Modification of the network morphology by RD and CTA.

During this study it turned out that photopolymers based on formulations II and VI are in best compliance with the properties of natural blood vessels.

Suture Tear Resistance Tests

To assess the suture tear resistance of the photopolymers designated for vascular tissue regeneration, a new test method was established. Molded specimens ($2 \times 10 \times 20\,mm^3$) were cut in the half with a knife (to avoid edge effects), provided with a seam of surgical suture (PP monofilament, suture size 6–0–0.07 mm in diameter) by the aid of a special gauge (seam distance 4 mm from the edge), clasped into a modified tensile testing arrangement (Figure 3 left) and loaded with a speed of 1 mm/min. A typical curve of the tear resistance test is shown in Figure 3 right.

The same method was applied for porcine coronary blood vessels to compare the results. The values of the tear resistance of the optimized formulation II already approach those of the natural blood vessels (Table 6).

Biocompatibility

Biocompatibility was assessed with in-vitro proliferation tests using human umbilical vein endothelial cells (HUVECs). In order to examine HUVEC attachment and growth on the photopolymer specimens developed in this study, the cells were

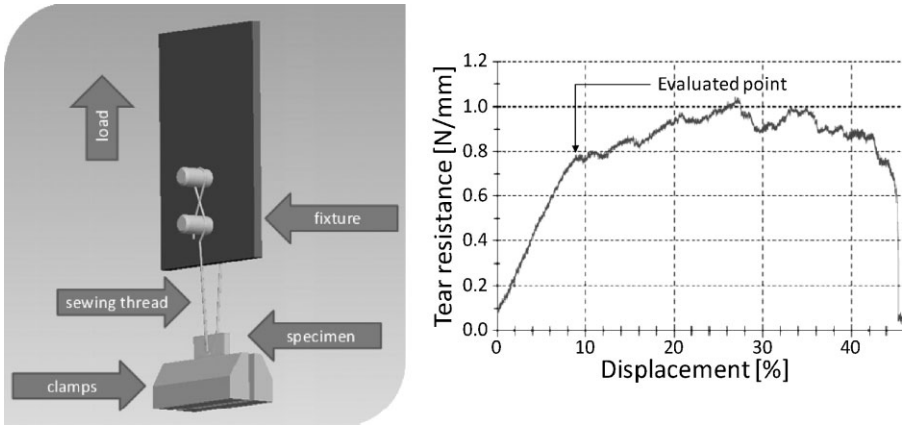


Figure 3.
Tear resistance test arrangement (left) and typical curve (right).

Table 6.
Results of the tear resistance tests.

Sample	Tear resistance [N/mm]
Formulation II	0.66 ± 0.06
Natural blood vessel	3.33 ± 1.33

seeded onto the disc-shaped test samples and special cell culture-treated plastic coverslips (proliferation controls) at a density of $4 \cdot 10^4$ cm^{-2} and allowed to adhere and proliferate for 24 h at 37 °C. Cell proliferation and morphology was examined by SEM (Figure 4). Both materials (II and VI) showed good results.

Shaping

To create artificial vascular grafts with a defined outer and inner structure it is necessary to apply powerful manufacture technologies. AM provides these methods and the possibilities involved. For this study the direct light processing (DLP, Figure 5 left) method was applied.[11] The photosensitive resin is irradiated from bottom-up through the window of the reservoir according to the 2D information of the current slice as a thin layer beneath the already printed part or the substrate respectively. This way the whole part is built up layer by layer. The first concept for shaping artificial blood vessels was to build a tube with a dense inner and a cellular outer wall (Figure 5, right). Figure 6 right depicts the successfully manufactured test structure out of the optimized formulation II.

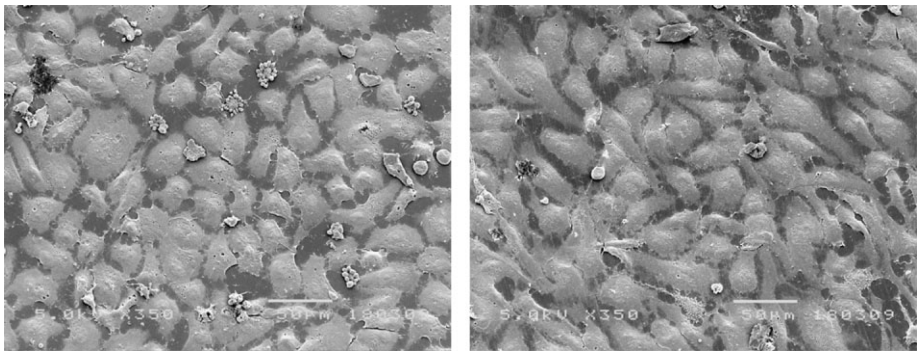


Figure 4.
SEM images of the cell culture tests of the II (left) and VI (right).

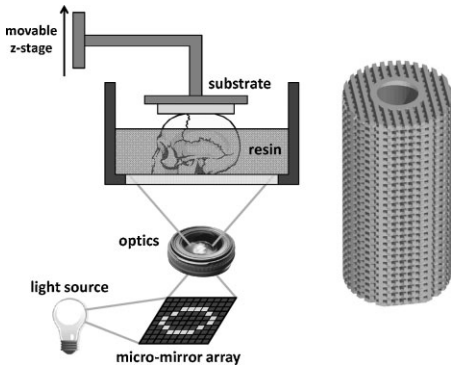


Figure 5.
Principle of DLP (left) and CAD of target structure (right).

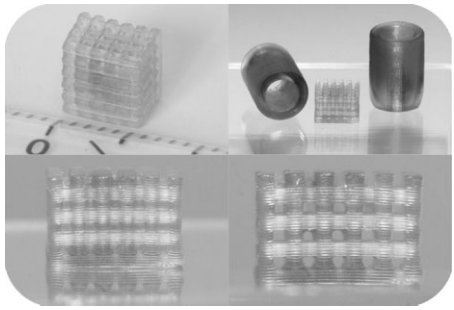


Figure 6.
3D-printed test structures of the optimized formulation II.

Conclusion

AM, especially DLP, is a suitable tool to manufacture cellular vascular grafts out of photopolymers. By the carefully selection of the different components for the photoresin it is possible to tailor the mechanical properties of the material. Particularly the application of the urethane based acrylate base monomer H1 in combination with the reactive diluent HEA und the chain transfer agent DOD provided very good results for the mechanical compliance, strength as well as biocompatibility of the photopolymers. Although the values of the tear resistance of these materials already approach those of natural blood vessel, there is still need for improvement. Finally it could be shown, that the optimized resin formulation can easily processed with the AM technology DLP to create arbitrary cellular structures.

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