Biocompatible and biodegradable photopolymers for microstereolithography

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The fabrication of geometrically well defined 3-dimensional scaffolds offering good cell adhesion is still an important topic in tissue engineering. Microstereolithography (µSLA), which is based on the photopolymerization of a liquid resin using a laser, turned out to be especially suitable because accessible resolutions of about 5 µm allow the fabrication of cellular structures for a number of biomedical applications. In order to find suitable materials the biocompatibility, determined by cell adhesion and cell proliferation of osteoblast-like cells, the photoreactivity, mechanical properties and cytotoxicity of several commercially available acrylate based monomers and polymers have been tested.

Despite the high rate of polymerization and the easy accessibility of a large number of monomers, the main drawback of these acrylate containing resins is the high cytotoxicity of residual unreacted acrylate groups. Therefore a new class of photoreactive monomers based on vinyl ester, carbonates and carbamates were synthesized. These materials showed lower cytotoxicity by a factor of 100 compared to similar acrylates the biocompatibility and mechanical properties of the polymers were evaluated, indicating the suitability of this new class of biophotopolymers for a number of applications in tissue engineering. Further tests regarding biodegradability of these polymers were performed.

Keywords: Additive Manufacturing Technology, Micro-stereolithography, biophotopolymers, biomaterials

1. Introduction

Additive Manufacturing Technolgy (AMT) techniques allow a material fabrication with defined geometry and pore structure. Microstereolithography (µSLA) has gained increasing interest over the last two decades and is often considered as one of the most innovative AMT techniques [1]. Therefore, it has become a very appealing methods for the production of three-dimensional scaffolds for several tissue engineering applications in the recent past [2],[3]. It is based on a layer-by-layer curing of a light-sensitive resin via photopolymerization. Radicals are being formed upon excitation of a photoinitiator by an UV-laser inducing polymerization and therefore, a phase transition of the resin from liquid to solid occurs. Materials solidification, through radical polymerization is most commonly based on acrylate or methacrylate chemistry [4]. Suitable biocompatible spacers for these type of reactive groups are based on alcohols like poly(ethylene glycol)[5],[6] and amines like chitosan.[7] It has to be noted that the main disadvantage of methacrylates is their limited reactivity due to the sterical hindrance and inductive stabilization of the formed radical of the additional methyl group.

Acrylates are considerably more reactive but also show a significant tendency towards Michael addition side reactions with amino groups of proteins or DNA giving hydrolytically non-cleavable aliphatic adducts. This frequently results in some significant skin irritancy or sometimes toxic effects making them very controversial for biomedical applications.

Hence, there is a huge need for other polymerizable groups that can be photopolymerized and structured with µSLA. Vinyl acetate is a radically polymerizable monomer showing low toxicity [8]. Poly(vinyl acetate) gives poly(vinyl alcohol) (PVA) as a degradation product upon hydrolysis, a water-soluble and biocompatible polymer that has been used for long-term implants [9]. However, vinyl acetate is a very volatile monomer, and therefore only higher molecular weight vinyl esters are eligible for µSLA. To keep PVA as polymer backbone and degradation product, vinyl carbonates and carbamates have been considered as well.

For basic investigations concerning the suitability of the vinyl based monomers for the photolithographic production of 3D scaffolds low molecular weight monomers with the same molecule size have been compared to (meth)acrylate references. Additionally a high molecular weight monomer was prepared to reduce the shrinkage of the eventual part built by µSLA (Fig. 1; adipinic acid divinyl ester (**4VE**), ethylene glycol divinyl carbonate (**4VC**), N,N'-dimethylethylenediamine divinyl carbamate (**4VCa**), 1,4-butandiol diacrylate (**4AC**), 1,4-butandiol di-

methacrylate (**4MA**), 3,6,9-trioxaundecanoic acid divinyl ester (**TVE**), tetraethylene glycol divinyl carbonate (**TVC**), ethylene glycol bis(3-aminopropyl) ether (**TVCa**), tetraethylene glycol diacrylate (**TAC**), tetraethylene glycol dimethacrylate (**TMA**) and trimer fatty acid trivinyl ester(**FVE**)).

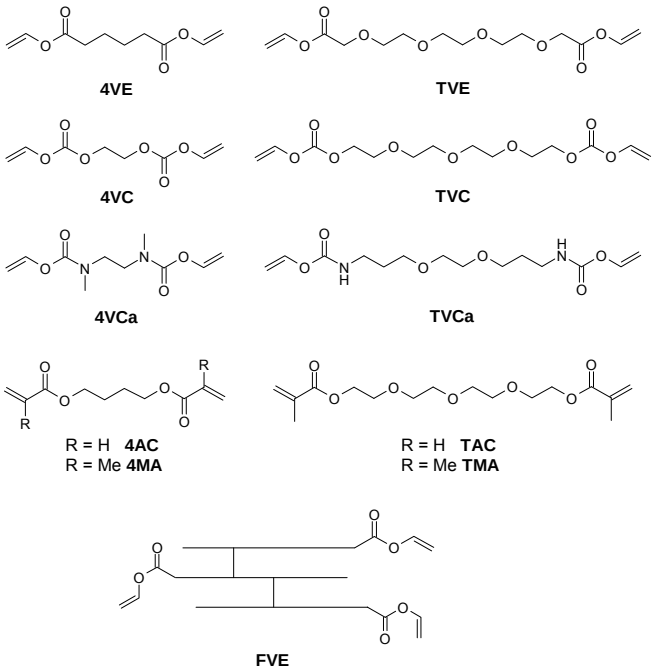


Figure 1 Vinyl monomers and (meth)acrylate references

To study the rate of hydrolysis hydrophilic tetraethylene glycol based monomers have been prepared and compared to (meth)acrylate references.

The monomers were tested with respect to their cytotoxicity in cell culture tests with osteoblast-like cells. Since photopolymerization is a prerequisite for a successful structuring, photoreactivity of the monomers were conducted by means of photo-differential-scanning-calorimetry (Photo-DSC). The mechanical properties of the resulting polymers were screened by nanoindentation to obtain an indentation modulus and hardness. For selected polymer mixtures the Young's modulus and flexural strength were determined by conventional 3-point-bending tests. Finally a suitable mixture should be selected for the 3D production of a cellular structure by µSLA.

2. Photo-Differential Scanning Calorimetry

Photo-DSC was conducted with a Netzsch DSC 204 F1. Photoreactivity of the monomers was tested by weighing approximately 8 mg of the sample into an aluminum DSC pan, which was subsequently placed in the DSC chamber. The samples were purged with a N_2 flow (~ 20 mL/min) for 5 min and irradiated with filtered UV-light (280-450 nm) from an Exfo OmniCure™ series 2000 with a light intensity of 3000 mW/cm^2 at the exit of the light guide. The measurements were carried out with 0.5 wt% (for the low molecular weight monomers) and 5 wt% (for the **FVE** containing formulations and the tetraethylene glycol) of bis(2,4,6-trimethylbenzoyl)-phenylphosphine oxide (Irgacure® 819, Ciba®) as photoinitiator at ambient temperature under nitrogen atmosphere. The samples were exposed to the light for at least 10 min, and the heat flow was recorded as a function of time. The double bond conversion (DBC) and rate of polymerization (R_p) were determined as described previously [10]. The DBC is an important factor for the practical application. Low values do not only lead to residual monomers or unreacted double bonds resulting in lower stiffness and strength of the material, they elevate the potential cytotoxicity of the material.

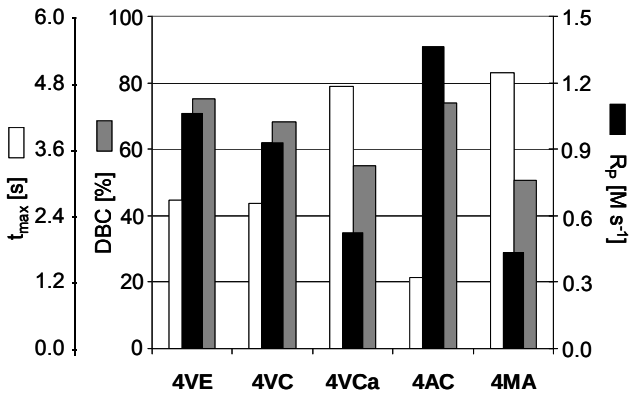


Figure 2 Photo-DSC of low molecular weight monomers

Figure 2 shows that among the low molecular weight monomers photoreactivity was found to be in the order following: acrylate (**4AC**) > vinyl ester (**4VE**) ≈ vinyl carbonate (**4VC**) > vinyl carbamate (**4VCa**) >methacrylate (**4MA**), expressed by a low t_{max} and a high R_P. Values for DBC increase in the same order, giving conversions between 64 % and 77 % for the new monomers. A DBC in that range is a typical value for highly crosslinked photopolymers due to limited diffusion at higher conversions.

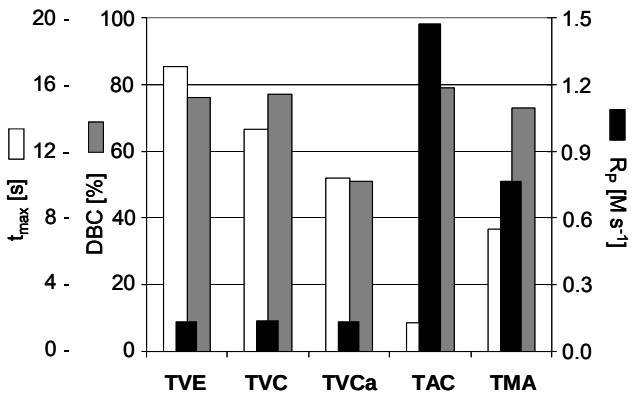


Figure 3 Photo-DSC of the tetraethylene glycol monomers

In contrast to the results obtained for low molecular weight compounds, the photoreactivity of the oligo(ethylene glycol) based monomers (Fig. 3) decreased in the order of acrylate (**TAC**) > methacrylate (**TMA**) > vinyl carbamate (**TVCa**) > vinyl carbonate (**TVC**) > vinyl ester (**TVE**). The lower reactivity compared to methacrylates might be explained by well-known chain transfer reactions of vinyl ester based monomers, which are favored in the presence of hydrogen abstractable domains like ethylene glycols.

3. Cytotoxicity

MC3T3-E 1 cells (donated by Dr. Kumegawa, Meikai University, Department of Oral Anatomy, Sakado, Saitama, 35002 Japan) were cultured in alpha MEM (Biochrom, Austria) supplemented with 4.5 g/L glucose, 5% fetal calf serum (FCS, Biochrom, Astria), and 10 µg/mL gentamycin. The cells were kept in humidified air under 5% CO_2 at 37°C. They were subcultured twice a week using 0.001% pronase E and 0.02% EDTA in phosphate-buffered saline (PBS).

For the experiments, the cells were seeded at a density of 20,000 cm^{-2} in 24-well culture plates and cultured over night. On the next day, the medium was changed and the cells were treated with decreasing concentrations of the monomers (10, 5, 2.5, 1.25 and 0.63 mM) for 5 days and compared to untreated cells. Thereafter, cell viability was addressed by incubation of the cultures with a colorimetric growth indicator based on the detection of cellular metabolic activity (EZ4U, Biomedica, Austria). Furthermore, the amount of DNA of the cultures was measured by fluorescence with Hoechst 33258 dye as a surrogate of the cell number. All experiments were performed as triplicate.

Table 1 Cytotoxicity of the monomers

Monomer	LC_{50} [mM]
4VE	1.5
4VC	> 10
4VCa	> 10
4AC	< 0.01
4MA	0.7
TVE	1.8
TVC	1.2
TVCa	0.8
TAC	< 0.01
TMA	0.5

The low molecular weight monomers and their tetraethylene glycol relatives gave values for LC_{50} in a range between 1-10 mM, as shown in table 1. Therefore, they are less toxic than methacrylate references by a factor of two and even up to two orders of magnitude less toxic than acrylates.

4. Degradation behavior

To study the influence of the new polymerizable groups on the rate of hydrolytic degradation, different model compounds for the polymers from methacrylates (ethyl pivalate), acrylates (ethyl isobutyrate), vinyl esters (isopropyl propionate), vinyl carbonates (ethyl isopropyl carbonate) and vinyl carbamates (isopropyl N,N- diethyl carbamate) have been prepared. These model molecules were dissolved in $EtOH/H_2O$ 3:1 as 0.1 N solutions. Degradation studies were carried out under accelerated conditions using 0.25 N NaOH, samples were taken and evaluated by GC using 1-octanol as a standard during the reaction (Fig.4)

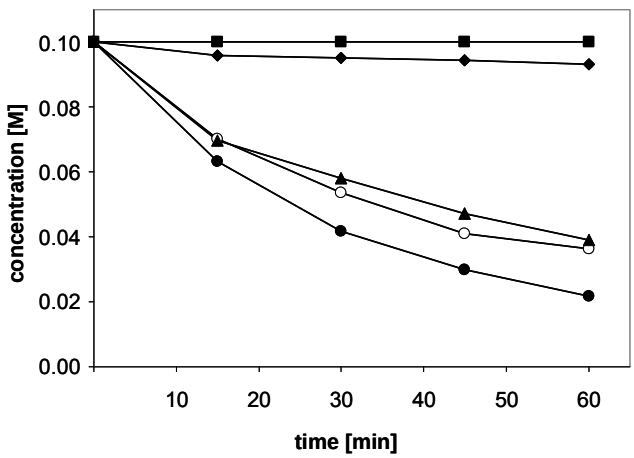


Figure 4 Degradation of model compounds: vinyl ester (●), acrylate (○), vinyl carbonate (▲), methacrylate (◆), vinyl carbamate (■)

Vinyl carbamates (~ 0 mmol/Ls) and methacrylates (16 mmol/Ls) seem to be nearly non-degradable, mainly due to the stable urethane group and the sterically hindered ester, respectively. Vinyl carbonates show the same degradation behavior as acrylates (~ 120 mmol/Ls) and vinyl esters decompose significantly faster (147 mmol/Ls), due the sterically less hindered carbon next to the carbonyl group compared to acrylates and especially methacrylates.

To investigate the hydrolytic degradation of polymer specimens, the ethylene glycol containing monomers have been compared to the corresponding (meth)acrylate and thermoplastic (PLA, PCL) references. The lower cross-link density and the higher hydrophilicity compared to the low molecular weight monomers make them significantly more susceptible to hydrolytical attack. Cylindrical polymer disks (5 mm in diameter, 1 mm in height) were placed into 2 mL of 1 N NaOH solution and the weight loss of the polymer was recorded over the time and the complete time of degradation extrapolated (Fig. 5).

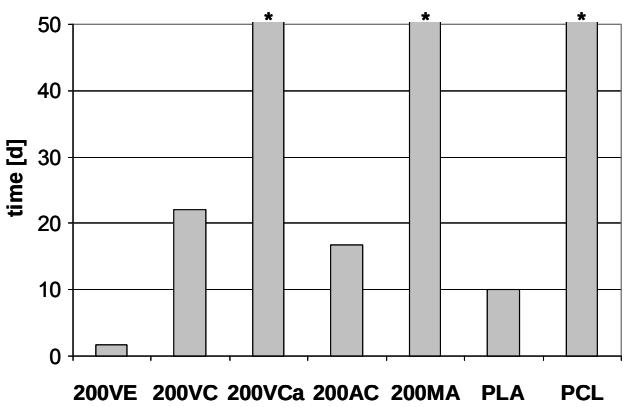


Figure 5 Time of complete degradation in 1 N NaOH

Correlating with the results obtained from the studies with the model molecules, degradation time of the polymers increases in order vinyl ester (**TVE**) < acrylate (**TAC**) ≈ vinyl carbonate (**TVC**) <methacrylate (**TMA**) ≈ vinyl carbamate (**TVCa**). The reference thermoplast PLA shows a complete degradation time between those

of **TVE** and **TVC**. For PCL, **TVCa** and **TMA** complete degradation could not be observed during the experiments.

5. Mechanical properties

Nanoindentation experiments were carried out on a Nanoindenter XP, MTS Systems Inc. Cylindrical specimens with 5 mm in diameter and a thickness of 1 mm were prepared in silicone moulds using 0.5 wt% of Irgacure® 819 (5 wt% in cases of the glycol based formulations) followed by photocuring for 5 to 30 min using a broadband UV lamp. Samples were then glued onto an aluminium cylinder with an epoxy-based adhesive and the surface was grinded and polished.

The specimens were indented with a velocity of 20 nm/s until an indentation depth of 2 µm was reached. From the recorded load versus displacement data indentation hardness (H_{IT}) and indentation modulus (E_{IT}) can be extracted [11],[12].

H_{IT} was calculated starting from the maximum force F_{max}. by applying Eq. 1 and Eq. 2:

$$H_{IT} = \frac{F_{\max}}{24.5 \cdot h_c^{\,2}} \tag{1}$$

$$h_c = h_{\max} - \varepsilon \cdot \left(h_{\max} - h_r\right) \tag{2}$$

where F_{max} is the maximum force in N, h_{max} is the penetration depth at maximum force in m, hr is the intersection of the tangent of the unloading curve at maximum load with the x-axis in m and ε an indenter constant.

E_{IT} was calculated starting from the slope of the unloading curve's tangent at the maximum load as shown in Eq. 3 and Eq. 4:

$$E_{IT} = \frac{1-(v_s)^2}{\dfrac{1}{E_r} - \dfrac{1-(v_i)^2}{E_i}} \tag{3}$$

$$E_r = \frac{\sqrt{\pi} \cdot S}{2 \cdot \sqrt{A_p}} \tag{4}$$

With v_s being the Poisson's ratio of the sample ($v_s = 0.35$), v_i the Poisson's ratio of the indenter tip (for diamond 0.07), E_r the reduced modulus of the indentation contact in MPa, E_i modulus of the indenter tip in MPa (1140GPa for diamond), S the contact strength in N/m (defined as the resistance of two particles against their mutual displacement) and A_p the projected area in m^2. At least seven measure points were taken for each sample.

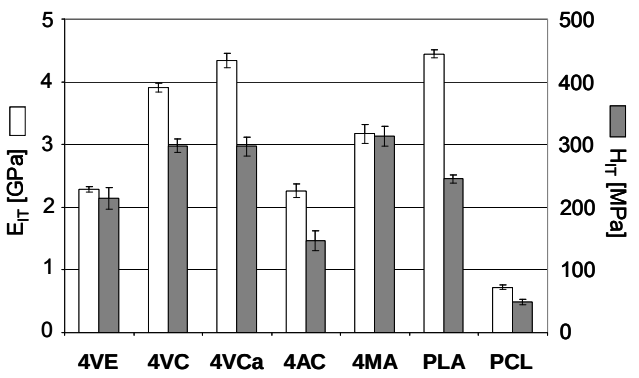


Figure 6 Nanoindentation of low molecular weight crosslinkers

All new monomers showed higher values for E_{IT} and H_{IT} than acrylate **4AC** and vinyl carbonate **4VC** and carbamate **4VCa** had even higher moduli than methacrylate **4MA** (Fig. 6), indicating sufficient curing through photopolymerization and therefore, confirming the results for double bond conversion in Photo-DSC experiments. All polymers were significantly stiffer than PCL and almost equal to PLA.

Tetraethylene glycol based polymers, as shown in Fig. 7, show qualitatively similar results for the influence of the different polymerizable groups on the mechanical properties.

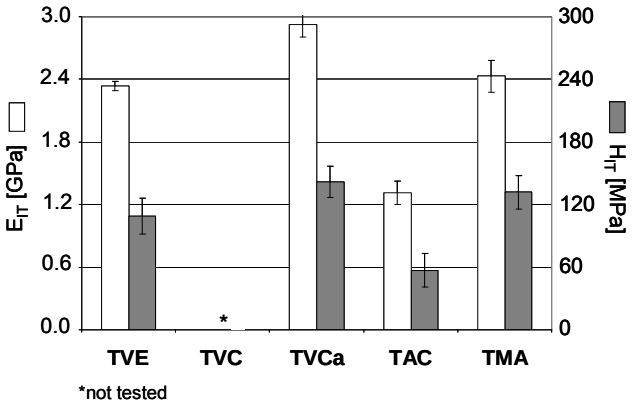


Figure 7 Nanoindentation of glycol based monomers

To evaluate mixtures of **4VE** with varying contents of the high molecular weight **FVE** to find the most suitable formulation for µSLA, 3-point-bending tests, obtaining the Young's modulus E and the flexural strength σ_{fB} were conducted.

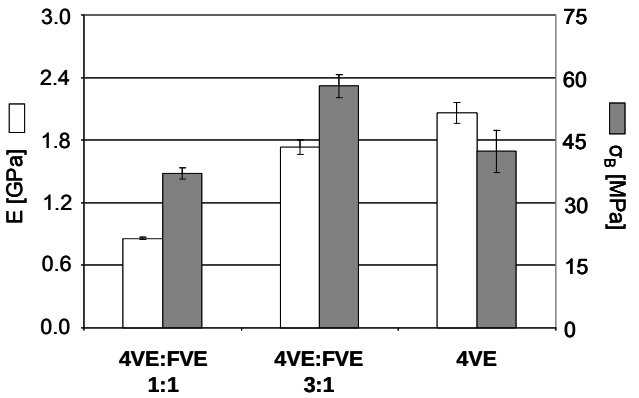


Figure 8 3-point bending tests of **4VE**/**FVE** mixtures

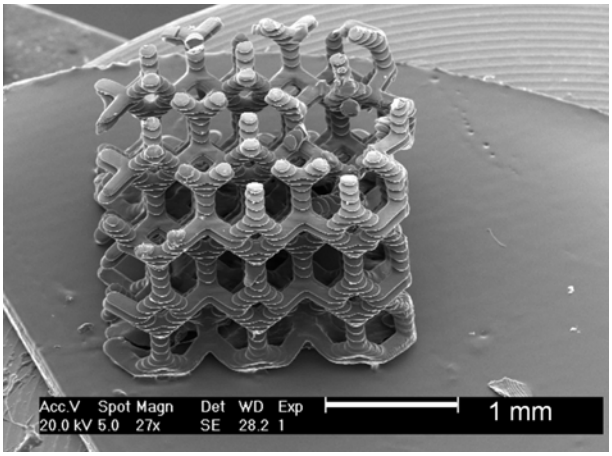
Mixtures containing three parts of **FVE** and one part of **4VE** had an indentation modulus of just 107 MPa and therefore, they were eliminated from the 3-point-bending tests (Fig. 8). The Young's modulus increases with higher content of the crosslinker **4VE** but the flexural strength seems to have a maximum for the **4VE**:**FVE** 3:1 polymer. From gross observations pure **4VE** seems to have the highest shrinkage leading to easier crack formation and higher brittleness, which explains the negative influence on the strength of the material. Correlating these findings with the results from the Photo-DSC experiments, **4VE:FVE** 3:1 seems to be the most suitable material for the shaping process by µSLA.

6. Microstereolithography

The fabrication of the 3D-parts was done using a µSLA system developed by the Laser Zentrum Hannover (LZH), as described previously (Fig. 9).[13] The utilized laser (Electronics 355 nm Quasi-CW Laser System XCYTE) was a neodymium doped yttrium aluminum garnet ($Y_3Al_5O_{12}$) laser (Nd:YAG) with a frequency multiplied wavelength of 355 nm (tripled), equipped with an acousto-optic modulator (AOM, Isomet AO Modulator RFA9x0-110 Series) and a scanner (SCANLAB hurrySCAN 14). The resin used contained a mixture of 4VE:FVE 3:1 with 3 wt% Irgacure® 819 and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (HMB) as an UV absorber.

Figure 9 µSLA device by LZH Hannover

The CAD model of the target structure was sliced into layers of 50 µm; the hatching and the line reduction were adjusted in order to obtain optimum results. The non-cured resin within the pores of the structure was rinsed with 2-propanol by using an ultrasonic bath before a post curing step under the UV lamp was performed. The resulting structure was examined by means of SEM (Fig. 10).



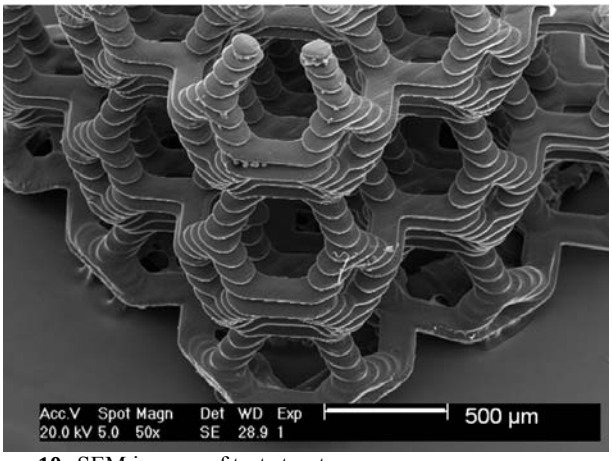


Figure 10 SEM images of test structures

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