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The Potential of Unsaturated Polyesters in Biomedicine and Tissue Engineering: Synthesis, Structure-Properties Relationships and Additive Manufacturing

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Abstract

The success of Tissue Engineering (TE) based approaches is strongly dependent on the development of novel biomaterials for the design of 3D matrices with tailored biomechanical properties to promote the regeneration of human tissues and organs.

This review covers the critical aspects related with the preparation of new unsaturated polyester (UP) resin formulations with suitable biological, chemical, thermal and morphological properties for the additive manufacturing (AM) of TE constructs. In this context, the basic principles of available AM technologies, with a special focus on novel stereolithography processes such as microstereolithography (micro-SLA), stereo-thermal-lithography (STLA), two-photon polymerization (TPP) and nanostereolithography (nano-SLA), are also presented and discussed. Ultimately, the present review will provide a better insight into the limitations and potential of combining UP and AM towards the rationale design/fabrication of complex artificial tissue substitutes.

Abbreviations
AA: adipic acid

AcA: acrylic acid

AIBN: α,α′-azoisobutyronitrile

AM: additive manufacturing

BM: 4,4-bismaleimidodiphenylmethane

BMP: butyl-3-mercapto propionate

BPO: benzoyl peroxide

BOD: biochemical oxygen demand

CA: *Candida Antarctica*

CAD: computer-aided design

CALB: Lipase B from *Candida Antarctica*

CAM: computer aided manufacturing

CDM: 1,4-cyclohexanedimethanol

CDRSP: Centre for Rapid and Sustainable Product Development (Portugal)

CHD: cyclohexanediol

CHMD: 1,4-cyclohexanedimethanol

ε-CL: ε-caprolactone

CTNB: carboxy terminated nitrile rubber

CQ: camphorquinone

DACC: 3,4-diacetoxycinnamoyl chloride

DAHs: dianhydrohexitols

DCC: dicyclohexylcarbodiimide

DEF: diethyl fumarate

DEG: diethylene glycol

DHCA: 3,4-dihydroxycinnamic acid

DHO: 6,7-dihydro-2(*5H*)-oxepinone

DMAMEA: dimethylamino methyl ethylacrylate

DMAP: dimethylaminopyridine

DMD: 2,2-dimethyl-1,3-propanediol

DMI: dimethyl itaconate

DMTA: dynamic mechanical thermal analysis

DN: double network

DSC: differential scanning calorimetry

EG: ethylene glycol

EO: ethylene oxide

EVA: ethylene vinyl acetate

FA: fumaric acid

FAME: fatty acid methyl ester

FU: diethyl fumarate

FUPR: fluorinated modified unsaturated polyester resin

GL: diethyl *trans*-glutaconate

Gl: globalide

HDA: 10-hydroxycaproic acid

HEMA: 2-hydroxyethyl methacrylate

HMA: *trans-β*-hydromuconic acid

HM: diethyl *trans-β*-hydromuconate

HPR: hydroxyl-ended hyperbranched polyester resin

HTNR: hydroxyl terminated natural rubber

HTPB: hydroxyl terminated polybutadiene

IA: itaconic acid

IM: isomannide

IS: isosorbide

IT: diethyl itaconate

MA: maleic anhydride

mCPBA: m-chloroperbenzoic acid

MEKP: methyl ethyl ketone peroxide

MH: 6-mercapto-1-hexanol

MM: *Mucor miehei*

MMA: methyl methacrylate

MPD: 2-methyl-1,3-propanediol

MPDO: 2-methyl-1,5-pentanediol

nACA: N-acetylcysteamine

NPG: neopentylglycol

NVP: *N*-vinylpyrrolidone

OMIS: oligo(isosorbide maleate)

PBF: poly(butylene fumarate)

PBSI: poly(butylene succinate-*co*-itaconate)

PC: *Pseudomonas cepacia*

PCL: poly(ε-caprolactone)

PCLF: poly(ε-caprolactone-*co*-fumarate)

PCL-PFPE-PCL: poly(ε-caprolactone)-perfluorpolyether- poly(ε-caprolactone)

PD: 1,2-propanediol

PEFS: poly(ethylene fumarate-co-sebacate)

PEG: poly(ethylene glycol)

PEGF: poly(ethylene glycol-*co*-fumarate)

PEGF-co-PHMCF: poly(ethylene glycol fumarate-co-hexamethylene carbonate- fumarate)

PET: poly(ethylene terephthalate)

P(FA-GLY-BA):   poly(fumaric acid-glycol-brassylic acid)

P(FA-GLY-DDDA):   poly(fumaric acid-glycol-dodecanedioic acid)

P(FA-GLY-PA):   poly(fumaric acid-glycol-pentadecanedioic acid)

P(FA-GLY-SA):   poly(fumaric acid-glycol-sebacic acid)

P(FA-GLY-TA):   poly(fumaric acid-glycol-tetradecanedioic acid)

PG: propylene glycol

PGA: poly(glycolic acid)

PGl: polyglobalide

PhA: phthalic anhydride

PHEMA: poly(2-hydroxyethyl methacrylate)

PHFS: poly(hydroquinone fumarate-*co*-sebacate)

PHMCA: poly(hexamethylene carbonate) diacrylate

PHV: poly(hydroxy valerate)

PHMCF: poly(hexamethylene carbonate-fumarate)

PRFS: poly(resorcinol fumarate-*co*-sebacate)

PLA: poly(lactic acid)

PLLA: poly(L-lactic acid)

P(MA-GLY-SA):   poly(maleic anhydride-glycol-sebacic acid)

PMMA: poly(methyl methacrylate)

PO: propylene oxide

PPF: poly(propylene fumarate)

PPFS: poly(propylene fumarate-*co*-sebacate)

PPS: poly(1,2-propylene succinate)

PSAGE: poly(3-allyloxy-1,2-propylene succinate)

PTSA: *p*-toluenesulfonic acid monohydrate

PU: polyurethane

ROP: ring opening polymerization

SA: succinic acid

SebA: sebacic acid

SEM: scanning electron microscopy

SLA: stereolithography

SN: single network

Sorb: Sorbitol

SPD: *(S)*-(+)-1,2-propanediol

STLA: stereo-thermal-lithography

TC: tamoxifen citrate

TE: tissue engineering

TEG: triethylene glycol

TGA: thermogravimetric analysis

THPA: cyclohex-4-ene-dicarboxylic anhydride

TMP: trimethylolpropane

TPA: terephthalic acid

TPP: two-photon polymerization

UM: unsaturated monomer

UPs: unsaturated polyesters

UPHR: unsaturated hyperbranched polyester resin

UPRs: unsaturated polyester resins

Table of Contents

1. Introduction

The history of polyesters dates back to the 1930's when Carothers reacted aliphatic diols with aliphatic diacids and established the relationships between the structure and properties of the obtained polymer. The results were not very promising since the polyesters obtained had low melting points, were very prone to hydrolysis, and no practical applications were found for the synthesized polymers. The search for polyesters with better properties continued and, in 1941, Whinfeld and Dickson, from Calico Printers Association, reported the synthesis of high melting and fiber forming polyesters from the reaction of terephthalic acid (TPA) and aliphatic glycols. The polyester that resulted from this work was poly(ethylene terephthalate) (PET), that is still today one of the most produced and used polymers worldwide [1-3].

Nowadays, polyesters constitute one of the most important and versatile classes of polymers, being suitable to be used in a variety of applications (*e.g.*, automotive industry, plastics industry, biomedical field, among others) [1, 3, 4]. Polyesters are prepared through a polycondensation process, where a dihydroxy compound (or a mixture of dihydroxy compounds) reacts with anhydrides or dicarboxylic acids [3, 4].

To achieve high reaction conversions and polyesters with high molecular weight the generated by-product, water, needs to be continuously removed from the reaction medium. For such purpose, a stream of nitrogen and vacuum can be used or, alternatively, the removal of water can be done with the help of solvents that are able to form azeotropes with water (*e.g.*, xylene or toluene). The use of catalysts is also common in the field of polyesterification. The most used are metal based catalysts (e.g., Sn(Oct)$_2$, ZnCl$_2$), and organic acids (*e.g.*, *p*-toluene sulfonic acid, PTSA) [5, 6]. The use of enzymes as catalysts has also been reported [7]. Ring opening polymerization (ROP) is another method that can be used to prepare polyesters. Typically, with this synthetic method it is possible to obtain polyesters with high molecular weight and low polydispersity (Đ) [1, 4]. Polyesters can be divided into different classes (*e.g.*, aromatic or aliphatic) depending on the type of monomers used. Two classes that result from this division are the saturated polyesters and the unsaturated polyesters (UPs). Examples of saturated polyesters are poly (ε-caprolactone) (PCL), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), as well as their copolymers. These polyesters found a broad range of applications in the biomedical field due to their biodegradability and biocompatibility. Some of the applications include scaffolds for tissue engineering [8, 9], sutures [10] and drug delivery systems [11]. Nevertheless, there is still a need to develop materials with more specific properties, for example, in terms of solubility, crystallinity, reactivity, among others [12]. Overall, this type of materials are hydrophobic and with few reactive groups along the backbone, thus limiting their applicability and further functionalization.

Regarding the UPs, these are characterized by their low-molecular weight and high polydispersivity values (Đ). The unsaturations present in the polymer backbone allow the UPs to be used in cure reactions via radical polymerization reactions, in the presence of an unsaturated monomer (UM), usually styrene, leading to thermosetting polyesters, known as unsaturated polyester resins (UPRs) [4, 13]. Maleic anhydride (MA) and fumaric acid (FA) are the most significant commercial monomers for introducing unsaturations in the polyester chain. The UP properties can be easily tuned by using different types of diols and diacids or even by changing the reactant ratio [13-15]. The properties of the UPRs depend not only on their molecular composition but also on the extent of the cure reaction, which means that it is very important to understand the reactions occurring during the curing and its implications in the process [15]. UPRs were developed in the 1930's, and still today are widely used in a variety of applications

closely related with the industry (*e.g.*, construction and automotive). Nevertheless, in the last years, efforts have been made to prepare UPRs more directed to biomedical applications [16-19]. In this case UPs are very attractive candidates mainly due to their unsaturations, providing further functionalization sites [20, 21] and enabling the fabrication of complex 3D structures via Additive Manufacturing (AM) processes [18]. This review intends to give a broad overview about the synthesis and preparation of UPRs, in particular the relationship between structure and properties as well as the latest developments regarding their application in the biomedical field. AM processes used for the production of TE scaffolds are reviewed with special focus on stereolithography (SLA).

2. UPs and UPRs: From synthesis to biomedical applications

2.1 The chemistry of UPRs

The transformation of UPs into a structural material requires the addition of another component, the UM, commonly named as diluent and generally used in weight percentages ranging from 30 to 40 % [13]. The generic process to obtain UPRs is divided in two main steps: the synthesis of the UPs and the curing reaction (Fig.1).

<Insert Figure 1>

The UM is added to the UP for two main purposes: (1) to reduce the viscosity of the system (aiding the resins processing) and (2) to create an efficient crosslink network with the double bonds available in the UP backbone. Styrene is the most commonly used UM, but others, like dimethacrylates, alkyl methacrylates vinyl esters, and divinylbenzene can also be used [22, 13]. Due to the radical nature of the curing process, the addition of a radical initiator to the formulation is needed. The most employed initiators are the organic peroxides, namely ketone peroxides, alkyl hydroperoxides, diacyl or dialkyl peroxides [4, 22]. Another important component of the formulation is the accelerator or promoter, a compound able to reduce the activation energy involved in the initiator decomposition, leading to the reduction of the temperature required for crosslinking. Vanadium or cobalt salts and tertiary amines

(*e.g.*, *N,N*-diethylaniline, *N,N*-dimethylaniline, or *N,N*-dimethyl-*p*-toluidine) are the most commonly used accelerators [4, 22]. Inhibitors, like hydroquinone or *t*-butyl hydroquinone, can also be used to avoid unwanted radical polymerization during the polycondensation (due to the high temperatures), mixture with UM, handling and storage. The amount of inhibitor, however, should be carefully adjusted to avoid further slowdown of the crosslinking reaction [4,13, 22].

When the UPR formulation is exposed to heating and/or radiation, the crosslinking reaction starts by the formation of free radicals from initiators. The first radicals formed are trapped by any inhibitor in the mixture until its full consumption. At this stage, the radical crosslinking reactions starts, forming long chain molecules through the connection of vinyl monomers, by intermolecular and intramolecular reactions (Fig 2A). These long chain molecules are predisposed to form spherical structures called microgels (Fig.2B). These gels can be defined as crosslinking dense structures, where several pendant groups (from UM) are confined to the interior of these structures [23]. This fact can result in the lowering of the final conversion, due to a chain segmental immobility in the crosslinked network. In the case of the reaction between UP and UM, previous studies demonstrated that gelation begins at an early stage of nearly 3-5% of conversion, which means that the reaction might be diffusion-controlled over almost its entire course [24]. Thus, the formation of microgel particles is a key feature of the UM-UP copolymerization. Yang and Lee [25] observed similar results in sheet molding compounds and in polyurethane-polyester interpenetrating polymer networks.

The curing process and the reaction kinetics are very complex due to the simultaneously occurrence of different reactions: UM-UP copolymerization, homopolymerization of UM and UP homopolymerization. The importance of each reaction can be adjusted depending on the formulation used and the curing conditions [26]. As shown in Fig.2A, these three processes can be divided into four possible reactions:

(1) Intermolecular crosslinking with (reaction I) or without (reaction II) linking between the UM;

(2) Intramolecular crosslinking with (reaction III) or without (reaction IV) linking through UM;

(3) Branching on the polyester molecule by UM (reaction V);

(4) UM homopolymerization (reaction VI).

<Insert Figure 2>

Reaction (1) results in a macroscopic network formation through the connection of adjacent polyester molecules; reaction (2) increases the crosslinking density but does not contribute to the macroscopic network formation; the other side reactions (3) and (4) may increase the polymer coil size, however their impact on the network formation is residual [25]. All these reactions affect the curing kinetics, but only the first two actually contribute to network formation.

Usually, the course of the UPRs cure is followed by differential scanning calorimetry (DSC) analysis [27-33].

2.2 Ways to tailor and improve the properties of the UPs and UPRs

Due to their excellent properties, easy synthesis, high versatility and low cost, UPRs applications continue to expand globally at robust rates. These type of materials had a leading role in the improvement of fiberglass reinforced products, for example in building materials for boats and cars [1, 3], as they are a very inexpensive and useful solution [15, 22]. UPRs are widely employed as adhesives and coatings but also in the building and electrical industries, among others [34]. Apart from the advantages, UPRs present also some disadvantages, such as their high flammability, low impact strength and poor toughness, which *a priori* can be mitigated by changing the UPR formulations. These changes can be performed in the structure of the UP, in the amounts or types of UM, or even in the initiator systems used.

Sanchez and co-workers [35] have studied the influence of styrene concentration in the final properties of the UPs using a commercial UPR, RESAPOL 10-203. The results provided by the dynamic mechanical thermal analysis (DMTA) showed a broadening of the tan δ peak as the concentration of styrene increased (from 6 %wt to 58 %wt). This fact was attributed to the existence of microenvironments that differ in their composition and crosslinking density. Above 18 wt% of styrene, two distinct transitions were observed and ascribed to the presence of two different and immiscible phases within the crosslinked network: an UP-rich phase and a polystyrene-rich phase. An increase in the T_g values was also reported for increasing contents of styrene. The sample with 38 %wt of styrene was found to be the most thermally stable.

It is known that when the UPs are prepared with only one diol, namely ethylene glycol (EG) or diethylene glycol (DEG), a limited solubility in styrene is observed. Nevertheless, Matynia and co-workers [36] managed to synthesize styrene soluble UPs making use of only one diol, EG, and two acid anhydrides, *viz.* MA and phthalic anhydride (PhA). The synthesized UPs were end-capped with isopropyl alcohol or monoesters from the used anhydrides, which enhanced their solubility in styrene. The increase in the amount of double bonds yields UPRs with high reactivity, high crosslinking density and higher mechanical stability. The UPRs obtained from the UPs end-capped with the monoesters showed better thermal stability and higher heat deflection temperatures than those end-capped with isopropyl alcohol.

The enhancement of toughness and impact resistance is a very important issue in the UPRs' field. One possible approach to achieve this goal encompasses the preparation of block copolymers of UPs incorporating rubber segments that are subsequently used in the preparation of UPRs. Cherian and Tachil [37] prepared copolymers of UP with hydroxyl terminated polybutadiene (HTPB), carboxy terminated nitrile rubber (CTBN) and also hydroxyl terminated natural rubber (HTNR). The polycondensation was carried out in two stages, leading to UPs containing alternating rigid and soft segments. The UPs were then crosslinked with styrene, in the presence of methyl ethyl ketone peroxide (MEKP), as initiator, and cobalt napthenate, as accelerator. The mechanical properties of the UPRs were evaluated and the results showed that CTBN is the most promising in improving the mechanical properties of the UPRs because the toughness and the impact strength were significantly enhanced, without jeopardizing extensively the remaining properties. Another approach was studied by the same researchers [38], in which poly(ethylene glycol) (PEG) segments were incorporated in the UP structure. The mechanical properties of the ensuing UPRs (35 %wt styrene) were evaluated and the influence of the incorporation of PEG in the UP structure was accessed. The results showed that the use of UPs with PEG segments of molecular weights *ca.* 200 g/mol resulted in UPRs with enhanced flexibility, fracture toughness and impact resistance. Following the same rationale, Cherian and co-workers [39] reported the use of polyurethane (PU) prepolymers as a mean to improve the mechanical properties of the UPRs. The prepolymers were prepared by reacting TDI with different polyols (HTNR, hydroxyl-terminated polybutadiene (HTPB), PEG and castor oil), and subsequently added in varying amounts (up to 10 %wt) to the UP to be crosslinked in the presence of styrene, using MEKP and cobalt napthenate as initiator and accelerator, respectively.

The results showed that the HTNR-PU prepolymer was the most effective in improving the mechanical properties of the UPRs. An increase of 20 % in the tensile strength was observed, while the toughness was shown to increase 188 %. Very important, the dynamic mechanical thermal analysis (DMTA) of the modified UPRs showed only one tan δ peak, indicating that the PU prepolymers are miscible with the UPR. In another approach, Rosa and Felisberti [40] incorporated poly(organosiloxane) segments in the UP network as a way to increase their flexibility.

The mechanical properties of UPRs have also shown to be improved by the addition of bismaleimides [41, 42], which are a class of compounds with two maleimide groups that are connected by a nitrogen atom via a linker, and are used as crosslinking agents in the polymer field. These molecules are known for their high thermal stability, strength, and fire resistance, among other interesting properties. Gawdzik and co-workers [42] proposed the modification of a commercial UP (D-1103) with 4,4-bismaleimidodiphenylmethane (BM). The authors reported that the addition of the BM accelerated the curing reaction, resulting in UPRs with high T_g values (*ca.* 180 ºC *vs* 70 ºC for the unmodified ones) and high hardness. It was also shown that BM can react with the UP at high temperatures, without any chemical initiator. When styrene is added to the system, different reactions can take place, namely UP-BM crosslinking, UP-styrene crosslinking, or even UP-(BM-styrene sequences) crosslinking, leading to UP moieties bridged by BM-styrene. Fig. 3 presents the structures of the BM and of the BM-styrene bridges.

<Insert Figure 3>

In a very interesting approach, Cherian and Tachil [43] studied how the addition sequence of the reactants in the UPs preparation influenced the final properties of the UPRs. The authors used five different sequences of reactants' (PhA, propylene glycol (PG) and MA) addition and found out that reacting first PhA with the whole amount of PG, with the subsequent addition of MA was the most promising sequence. This procedure led to shorter reaction times, and the resulting UPs yield UPRs with enhanced elongation at break and toughness.

Fluorinated polymers, in turn, have shown to be suitable to improve both the mechanical properties and surface properties of the UPRs, as demonstrated by Messori and co-workers [44]. The authors reported the modification of an UPR by blending it with poly(ε-caprolactone)-perfluoropolyether- poly(ε-caprolactone) (PCL-PFPE-PCL)

triblock copolymers. The fluorinated modified UPR (FUPR) showed a surface enrichment in fluorine segments and, as expected, a decrease in the water diffusion coefficient value. The morphology of the FUPR has shown to be strongly dependent on the molecular weight and the PFPE/ PCL ratio. It was also found that a plasticization effect occurred with an increase in the molecular weight of the triblock copolymer and in the PCL length. The absorbed energy at break was observed to be enhanced when the PFPE/PCL ratio was 2/10.

In another work, Nebioglu and co-workers [45] studied the effect of the amount of both multifunctional UM and internal and terminal unsaturation in the network structure and in the mechanical properties of the resulting UPRs. Acrylate-terminated UPs were obtained from the reaction of MA, adipic acid (AA), neopentyl glycol (NPG), and trimethylolpropane (TMP), and their end-chains were modified with acrylic acid (AcA). The UPRs were obtained from the photopolymerization of the UPs in the presence of trimethylolpropane triacrylate (TMPTA) as UM, and Irgacure 184 as photoinitiator. The DMTA showed that both high concentrations of TMTPA and high internal unsaturation led to phase separation in the cured UPRs. It was also observed a broadening of the tan δ curve, revealing the heterogeneity of the sample, as the amount of TMTPA increased. Surprisingly, the increase in the amount of internal and terminal unsaturations was not translated in an improvement of the crosslinking density. The authors attributed this result to the fact that a higher unsaturation concentration causes more microgelation, resulting in a higher amount of trapped free radicals inside the microgels, with consequent reduction in the crosslinking density. The microgels were also responsible for a decrease in the fracture toughness properties and on the reverse impact resistance. In a subsequent work, the authors [46] used the same type of UPs to obtain UV-cured films and studied how the extent of microgelation affected the viscoelastic, fracture and tensile properties of the films. The results demonstrated that when the microgelation occurred in a low extent, the microgels acted as micro-support units enhancing the mechanical properties. However, for high extent of microgelation, a phase separation occurred jeopardizing the mechanical properties of the films.

Another very important aspect in the field of UPRs is related with their thermal stability. Some strategies regarding the improvement of this property have been presented in literature. Tawfik [47] developed different UPs based on durene derivatives, *viz.* 3,6-bis(methoxymethyl)durene and 3,6-bis(benzyloxymethyl)durene (Fig. 4A), that were obtained from the 3,6-bis(chloromethyl)durene. The other

monomers used in the preparation of the UPs were PhA, MA and succinic acid (SA) as well as different glycols, such as PG, triethylene glycol (TEG), and cyclohexane diol (CHD). The UPs were able to cure with styrene, at room temperature, in the presence of cobalt naphthenate and MEKP as accelerator and initiator, respectively. From the thermogravimetric analysis (TGA) and DSC studies, the authors found that the UPR prepared from UP V (see Fig. 4B) was the resin with the highest thermal stability. Additionally, both this UPR and that obtained from UP III (see Fig. 4B) showed flame-retardant properties.

<Insert Figure 4>

In a different approach, Tibilleti and co-workers [48] improved the thermal stability and fire behavior of UPRs through the introduction of nanometric alumina oxide and submicron alumina trihydrate.

Alkskas and co-workers [49] developed thermally stable UPs containing a cyclopentapyrazoline moiety in the main chain. The cyclopentapyrazoline moiety is known to enhance the thermal stability of the materials, their solubility and can also provide the materials with photoconductivity. The method for the preparation of the UPs was based on an interfacial polycondensation between diols bearing the cyclopentapyrazoline moiety, namely 3-p-hydroxyphenyl-6-p-hydroxybenzylidene cyclopentapyrazoline and 3-anillyl-7-vanillylidene cyclopentapyrazoline, and different diacyl chlorides, *viz.* adipoyl, sebacoyl, isophthaloyl, and terephthaloyl dichlorides (Fig. 5).

<Insert Figure 5>

The UPs presented T_g values ranging from 103 ºC to 208 ºC, being soluble in a variety of organic solvents. Thermal analysis showed that the aromatic based polyesters were more thermally stable than the aliphatic counterparts. It should be stressed, that this new UP also showed electrical conductivity when doped with iodine. Unfortunately, no data regarding the cure reaction of this UP to yield the UPRs was reported by the authors.

The synthesis of modified UPs to enable the control of product viscosity was performed by Chiu and co-workers [50]. The authors grafted thermally breakable functional groups based on diketogluconic acid onto the UPs backbone and the results showed that these moieties were crucial to control the viscosity during the moulding process.

In another work related with the viscosity control, Zhang and co-workers [51] developed new unsaturated hyperbranched polyester resin (UHPR) based on the reaction between MA monoisooctyl alcohol ester and a hydroxyl-ended hyperbranched polyester resin (HPR). The hydroxyl ended HPR was prepared from PhA and TMP. The UHPR showed a viscosity below 10,000 cP, at room temperature, being suitable to be used in the field of coatings.

It is known that UPRs can have large volume shrinkage during the polymerization, which leads to sink mark formation, surface waviness, warpage, poor dimension accuracy, or internal crack. This particular aspect can pose several issues to the application of these materials in AM processes. Shenoy and co-workers [52] reported a study on the effect of the addition of ethylene vinyl acetate (EVA) (partially depolymerized and presenting different degrees of branching) on the mechanical, thermal, chemical and shrinkage properties of UPRs. The authors concluded that the addition of 0.5 % wt EVA (with a high degree of branching) led to an improvement in the flexural and tensile properties, without deleterious effects in the HDT and impact properties. A decrease in the percentage of shrinkage of ca. 2% was observed for the system containing 0.5 % wt EVA. Further increase in the EVA percentage led to a decrease in the volume shrinkage, but a notorious worsening of the remaining properties was observed. It is known that the increase of the crosslinking density results in a decrease of shrinkage behavior. This phenomenon will not be fully discussed here, although it assumes a great importance in industrial fields and has been intensively investigated [53-55].

Another important aspect in the UPRs field is related with the initiator systems that are used. As mentioned before, tertiary amines are widely used as accelerators, but often the formulations containing these compounds have a reduced shelf-life. A possible strategy to overcome this issue is to incorporate the amine in the UP structure [56-59]. For such purpose, Duliban [56] developed novel amine modifiers with hydroxyl terminal groups to be incorporated in the UPs. The amines were prepared from the reaction of ethylene oxide (EO) or propylene oxide (PO) with *N,N'*-diphenylethane-1,2-diamine or from the reaction of EO with *N,N'*-diphenylhexane-1,6-diamine (Fig. 6). The amine modifiers were used to partially replace propylene glycol (PG) (up to 2 % mol) in a UP composed by MA, PhA, PG and DEG. The UPs were cured in the presence of benzoyl peroxide (BPO), the most used initiator in amine-curing systems, or alternatively in the presence of the initiator methyl ketone hydroperoxide and the accelerator cobalt(II) octanoate.

The results obtained from the cure reactions showed a reduction of the gelation time in both curing systems when 2 %wt of the modifier was used. The best result, in terms of gelation time and storage stability, was obtained for the UP containing 1 % wt of the modifier 3,6-diaza-3,6-diphenyloctane-1,8-diol. The addition of benzyltriethylammonium chloride to the cobalt curing system was also performed as a way to improve the storage stability of the resins. However, the stabilizer reduced the resins reactivity.

<Insert Figure 6>

In the same line of research, Kucharski and co-workers [59] prepared diols bearing tertiary amine groups, from the reaction of *N,N*-dimethyl-*p*-phenylenediamine with EO or PO. The results showed that when the amine diols are incorporated in the UP structure, a reduction in the gelation time of the respective UPRs is observed. Nevertheless, the UPRs stability is also compromised exhibiting a significant reduction. Related works by Duliban [57, 58] show that amine diols (or polyols) bearing *s*-triazine rings (Fig. 7), when introduced in the structures of the UPs, are also effective in reducing the gelation times of the respective UPRs.

<Insert Figure 7>

The catalyst of polycondensation to prepare the UPs can also be modified as a route to improve the efficiency of the reaction. In this sense, Alemdar and co-workers [60] proposed a boric acid-pyridine mixture as a mild catalyst for the preparation of UPs, from hydroxyethyl esters *in-situ* generated from cyclic anhydrides (maleic, succinic and phthalic anhydrides) (Fig. 8). The UPs, with molecular weights ranging from 1650 to 1950 g/mol, were obtained after 4h of reaction. This reaction time can be seen as a great advantage since it opens the possibility of avoiding side-reactions (*e.g.*, branching or crosslinking from the reaction of hydroxyl groups with the double bonds). It was also found that the UPs easily bind to sodium bisulfite to give sulfonated derivatives that have an amphiphilic nature.

<Insert Figure 8>

In another work, Lou and co-workers [61] demonstrated that it is possible to obtain UPs with a controlled structure and tailorable properties by ROP. The homopolymerization of 6,7-dihydro-2(5*H*)- oxepinone (DHO) and its copolymerization with *ε*-caprolactone

(ε-CL) [40] were initiated by aluminium isopropoxide [Al(O^{i}Pr)$_3$], in toluene, at room temperature (Fig. 9).

<Insert Figure 9>

The poly(DHO) was obtained with the predicted molecular weight, with a narrow molecular weight distribution, and with T_g and T_m of -50ºC and 35 ºC, respectively. The copolymers DHO with ε-CL were also prepared in a controlled manner. From this experiment it was also possible to observe that an increase in the DHO amount in the random copolymer increases the T_g and decreases the T_m, in comparison with PCL.

2.3 Alternative crosslinking methods for the UPs

Commonly, the UPRs are obtained from the cure reaction between the UP and the UM. Due to its low cost and availability, styrene is often used as UM. Additionally, styrene is known to provide UPRs with excellent mechanical properties. However, this UM has high volatility, and during the cure process it is released to the atmosphere. Thus, in the recent years, significant efforts have been devoted to find alternative UMs capable to overcome this problem. Possible strategies encompass the use of UMs with boiling temperatures higher than styrene [62, 63], the reduction of styrene content in the formulation and, more interesting, the introduction of different moieties in the UP structure to allow the further crosslinking in the absence of UM. It should be noted, that in this subsection the described strategies are exclusively related with fossil based UPRs. The strategies focused on the development of self-crosslinkable biobased UPs will be presented in the following section of the manuscript.

In a very interesting contribution, Straub and co-workers [64] developed alternative crosslinking methods for UPs avoiding the use of styrene. The strategy proposed was based on the use of maleic or fumaric diesters functionalized with triple bonds, followed by oxidative metal mediated homo-coupling of the introduced alkyne moieties (Fig. 10). The authors discovered that the combination of Pd/Cu and air is a very effective system for the catalytic oxidative homo-coupling of alkynes, leading to the crosslinking of UPs chains.

<Insert Figure 10>

In turn, Cinar and co-workers [65] proposed the use of bis(nitrone)s as crosslinkers for UPs bearing maleate and fumarate groups. The nitrones are known to easily undergo 1,3-cycloadditions with double bonds. The crosslinking reaction successfully occurred at 120 ºC, for 30 min, in the absence of catalysts (Fig. 11). Unfortunately, no data regarding the properties of the UPRs were presented by the authors.

<Insert Figure 11>

Worzakowska [34] reported the synthesis of UPs through polycondensation of cyclohex-4-ene-dycarboxylic anhydride (THPA), MA and different diols (1,4-butanediol, 1,6-hexanediol and EG), as well their selective epoxidation with peracetic acid in mild conditions (Fig. 12) [66], to yield unsaturated epoxyoligoesters. It should be noted that using this method only the double bonds in the cyclohexenyl ring were converted in epoxy groups, leaving the remaining C=C bonds (from the maleate units) unmodified. The ensuing polymers have the ability of being crosslinked in the presence of UMs or by polyaddition reactions with suitable curing agents. Interestingly, the resins obtained from the cure of unsaturated epoxyoligoesters in the presence of 20 %wt styrene (usually, the percentage of styrene is about 37 %wt), and using BPO as initiator, revealed a better thermal stability and increased viscoelastic properties in comparison to those obtained from the unmodified UP. The properties were further improved when THPA was added to the curing system. In this case the crosslinked network resulted from two types of reaction: (i) copolymerization of the C=C double bonds in the polyester and the UM, and (ii) polyaddition of epoxy groups to anhydride groups, forming additional diester segments. The length of the diol has demonstrated an important influence in the final properties of the resins; shorter diols led to resins with better properties than those obtained with larger diols.

<Insert Figure 12>

2.4 UPs and UPRs from renewable monomers

A field that is nowadays attracting considerable attention from the scientific community is the preparation of polymers from renewable resources [67, 68]. In the last years,

many contributions regarding the use of renewable monomers (e.g., FA, 2,5-furandicarboxylic acid (FDCA), itaconic acid (IA), sebacic acid (SebA), dianhydrohexitols (DAHs), 1,3-propanediol [16-18, 69-71], or vegetable oils [72-74]) in the preparation of UPs and, ultimately, UPRs have been reported. For a better understanding, the first part of this subsection will be devoted to the preparation of biobased UPs, where some approaches regarding the crosslinking reactions of these polymers in the absence of UM will be presented; the second part will be focused on the development of biobased UPRs, resulting from the crosslinking reaction of biobased UPs with UMs.

Takenouchi and co-workers [75] prepared a set of UPs based on different anhydrides (succinic, methylsuccinic, maleic and citraconic), FA, and EG and studied the effect of the geometric configuration (*E*- and *Z*-) of the double bonds in their biodegradation properties. Enzymatic degradation tests were carried out at controlled temperature (37 ºC) in the presence of *Rizopous delemar* lipase. Additional degradation tests were carried out with an activated sludge (25 ºC) and the values of the biochemical oxygen demand (BOD) registered. The overall results showed that the UPs with the double bonds in the *E*-configuration are more prone to degradation, when compared to those that have the double bonds in the *Z*-configuration. It was also found that the methyl group as a pendent chain contributes to a decrease in the biodegradability of the samples.

Zheng and co-workers [76] synthesized multiblock copolymers based on poly (butylene fumarate) (PBF) and poly (1,2-propylene succinate) (PPS), by the chain extension reaction reaction of PBF diol and PPS diol with 1,6-hexamethylene diisocyanate (HDI) (Fig. 13). The main purpose of this work was to increase the biodegradability of PBF by introduction of PPS moieties.

<Insert Figure 13>

The results showed that the biodegradability of PBF was enhanced by the copolymerization of PPS. For contents above 20 %wt, the effect is more notorious. Also, the introduction of PPS led to materials with higher impact strength and less crystallinity when compared with PBF.

Najafi and Sarbolouki [77] prepared partially water-soluble UPs through the reaction of two linear unsaturated aromatic oligoesters, *viz.* poly(hydroquinone fumarate-co-sebacate) (PHFS) and poly(resorcinol fumarate-co-sebacate) (PRFS), and PEG. Both

diblock (PHFS-*co*-PEG and PRFS-*co*-PEG) and triblock copolymers (PEG-*co*-PHFS-*co*-PEG and PEG-*co*-PRFS-*co*-PEG) were prepared and their thermal properties were assessed. It was found that both the diblock and triblock copolymers were thermally more stable than the unsaturated oligoesters. The DSC analysis showed that the block copolymers had one exothermic transition, corresponding to the melt of the PEG segment. The same authors [78] also reported the preparation diblock and triblock copolymers from poly(propylene fumarate-*co*-sebacate) (PPFS), poly(ethylene fumarate-*co*-sebacate) (PEFS), and PEG. Similarly to what was previously reported, the copolymerization of the unsaturated oligoesters with PEG results in an increase of the thermal stability. The *in vitro* hydrolytic degradation (pH=7.4, 37 ºC) was evaluated and the ^{1}H NMR analysis revealed that the fumarate ester bond cleaves faster than the ester sebacate bonds.

Jasinska and Koning [79] also reported the preparation of water-soluble UPs making use of PEG, isosorbide (IS), and MA. The bulk polycondensation, catalyzed by titanium(IV) n-butoxide was the chosen synthesis method. The results have demonstrated that these UPs presented branched structures resulting from the reaction of the double bonds with the –OH groups of the PEG or IS moieties. The thermal properties of the UPs were evaluated and the results showed that the T_g was dependent on the amount of PEG in the structure; for higher contents of PEG, lower T_g were observed. The UPs were thermally stable up to temperatures of 255 ºC ($T_{5\%}$). The authors suggested that these UPs could be suitable for coating applications.

Although the polycondensation reaction catalyzed by metals is the common method used in the industry and in most academic reports dealing with the preparation of UPs, in the last years the enzyme-catalyzed polycondensation has gained increasing importance.

Olson and Shears [12] prepared unsaturated aliphatic polyesters by polycondensation of *trans-β*-hydromuconic acid (HMA) with several diols (C_4 to C_{10}, and DEG) using enzyme (Lipase B from *Candida Antarctica* (CALB)) and metal based catalysts (Fig. 14). The use of AA as the second diacid was also tested. HMA was the selected unsaturated diacid because it is not prone to the side reactions commonly associated with FA. The UPs prepared from this monomer usually present higher molecular weights. The UPs resulting from the reaction of HMA with the different diols were obtained in high yields, with molecular weights between 2500 to 10500 g/mol, and Đ below 2. The highest molecular weight was obtained from the enzyme catalyzed

reaction of HMA with the C_8 diol. The UP also showed to be thermally stable up to temperatures of approximately the 320 ºC. When AA was used as comonomer, the enzyme catalyzed reaction has also shown to be effective in producing UPs with higher molecular weight when compared with those obtained from the metal-catalyzed reaction, for the same reaction time (13000 g/mol vs 11600 g/mol). The T_m of all the prepared UPs was found to be dependent on the length of the diol, being higher when diols with longer aliphatic chains were used. Another UP, from DEG and HMA, was also prepared, being liquid at ambient temperature with a T_g of -40 ºC.

<Insert Figure 14>

In another contribution, Naves and co-workers [80] reported the polycondensation of IS or isomannide (IM) with diethyl adipate and different unsaturated diesters, *viz.* diethyl fumarate (FU), diethyl itaconate (IT), diethyl *trans*-glutaconate (GL) and diethyl *trans*-β-hydromuconate (HM) (Fig.15), using CALB as catalyst.

<Insert Figure 15>

The UPs based on IM showed higher molecular weights, but lower isolated yields, than their IS based counterparts. The UP with the highest molecular weight was obtained when FU was used as the unsaturated diacid; a decrease in the UPs' molecular weight was observed for increasing chain lengths of the unsaturated diacid. The same method of synthesis was used with less sterically hindered diols, namely 1,4-butanediol and 1,6-hexanediol, and it was found that the molecular weights obtained were very close to that presented by the UPs prepared from IS and IM. The MALDI-Tof analysis showed inequivocally the presence of the double bonds, making the materials good candidates to be used in crosslinking reactions.

Jiang and co-workers [70] synthesized biobased UPs by the polymerization of diethyl succinate, dimethyl itaconate and 1,4-butanediol in solution, by a two-stage method (Fig. 16), in the presence of CALB.

<Insert Figure 16>

The use of diphenyl ether as the solvent allowed the preparation of UPs with higher molecular weight, when compared to that obtained with the other used solvents (diglyme and dodecane). The best result in terms of molecular weight (*ca.* 13000 g/mol) and yield (90%) was obtained for the UP prepared with a molar feed amount of

IT of 15 %. The NMR analysis showed that the amount of double bonds can be easily changed by using different amounts of dimethyl itaconate (up to 35 % mol). The results provided by the thermal analysis showed that the amount of dimethyl itaconate had no significant influence on the T_g (a variation of 3ºC was observed) or on the thermal stability. In turn, the T_m revealed an almost linear decrease, with the increasing amount of dimethyl itaconate (from *ca.* 115 ºC to 70 ºC).

The same research group [71] reported the enzymatic synthesis, using the same enzyme, of poly(butylene succinate-co-itaconate) (PBSI) making use of three different methods: two-stage melt polymerization, azeotropic polymerization using cyclohexane/toluene as solvent, and two-stage solution polymerization in diphenyl ether. The authors reported that when the dialkyl esters (dimethyl itaconate and diethyl succinate) were replaced by the respective dicarboxylic acids (IA and SA) only oligomeric species were obtained. When using dialkyl esters, the best results in terms of molecular weight and yields were obtained with the azeotropic distillation method. In this case, it was possible to use molar feed amounts of dimethyl itaconate up to 50 %. As previously noted [70], a decrease in the crystallinity of the UP was observed with increasing amounts of the itaconate moieties. The UPs were crosslinked via a hot press method, in the presence of dicumyl peroxide. The resulting crosslinked materials have shown to be brittle, presenting high tensile strain and low rupture strain.

Very recently, Jiang and co-workers [69] enlarged the library of biobased UPs, making use of the two-stage enzymatic polycondensation (catalyzed by CALB) previously reported [71, 70]. The authors prepared various polyesters using 1,4-butanediol, dimethyl itaconate and diacid ethyl esters with different chain lengths. The results showed CALB presents the highest specificity for diethyl adipate, among all the tested diacid ethyl esters. The thermal properties showed to be dependent on the length of the diacid ethyl ester used. The UV curing reaction was performed using Irgacure 184 and the thermomechanical properties (T_g, Young modulus', tensile stress, and rupture strain) of the cured film could be easily adjusted and tuned by controlling the amount of itaconate moieties in the UPs structure.

Another work, reported by Tsujimoto and co-workers [81], used enzymatic polymerization between divinyl esters and glycerol in the presence of unsaturated fatty acids to develop biodegradable crosslinking polyesters. In this study different lipases were tested as catalyst, namely, *Candida Antarctica* (CA), *Mucor miehei* (MM), and *Pseudomonas cepacia* (PC). The comparison between the activity of the three lipases,

revealed that CA lipase was the best in promoting the polycondensation. The UPs were able to be crosslinked in the absence of UM, using two methods: (i) catalytic oxidation in air, using cobalt naphthenate, and (ii) thermal treatment (150ºC, 2 h). The film obtained from the UP made from divinyl sebacate and linoleic acid was subjected to the BOD test in an activated sludge. The results highlight the gradual degradation of the film, and after 42 days the percentage of biodegradation of the film was 45%. The same enzymatic polymerization was used to synthesize polyesters based on divinyl sebacate, glycerol and unsaturated fatty acids or epoxidized fatty acids [82]. The polyesters bearing the unsaturated polyesters were further epoxidized in the presence of CA lipase. This work showed that the enzymatic polymerization can be carried out using both unsaturated and epoxidized moieties. The crosslinking reaction was carried out as previously presented [81] and the biodegradability of the films (assessed by the BOD test) showed a percentage of biodegradation near 50%, after 50 days of test.

Very recently, Goerz and Ritter [83] crosslinked biobased UPs made from IA, IS and SA in the presence of N-alkylated dinitrones based on IS (Fig. 17). The results obtained showed that the new dinitrones were very effective in promoting the crosslinking of the biobased UPs via a 1,3-dipolar cycloaddition.

<Insert Figure 17>

Another important class of renewable monomers that has been used in the development of UPs are the cinnamic acid derivatives. These compounds are able to undergo [2+2] cycloaddition reactions under UV irradiation (λ>260 nm), forming photoreversible crosslinks. Thus, the polymers comprising these derivatives in their structure are photoresponsive [84, 85]. Dong and co-workers [86] developed new biodegradable polyesters by the polycondensation reaction of 3,4-dihydroxycinnamic acid (DHCA), obtained from lignin, and 10-hydroxycaproic acid (HDA), obtained from the castor oil (Fig. 18). The authors evaluated how the properties of the UPs were affected by the amount of each monomer in the polymer chain. An increase in the amount of HDA led to more flexible materials, and was also shown to improve the elongation at break of the UPs. The UPs were further crosslinked by UV irradiation (λ>320 nm) and their mechanical properties were also studied. The crosslinked UPs showed improved tensile strength, but low elongation at break. The *in vitro* hydrolytic degradation tests revealed

that the UPs were able to be degraded. Surprisingly, the degradation rate was higher for the crosslinked UPs.

<Insert Figure 18>

The preparation of copolymers based cinnamic acid derivatives and LA [87-89] have also been reported, and different approaches have been used. In a very interesting report, Thi and co-workers [88] used the –OH terminal group of PLLA to attach 3,4-diacetoxycinnamoyl chloride (DACC), to yield DACC-PLLA. The ensuing polymers showed excellent thermal stability when compared to neat PLLA (an increase of about 100 ºC in the thermal stability was observed). Also, the DACC-PLLAs revealed the ability to undergo [2+2] cycloaddition reactions when irradiated with UV light with λ>280 nm (Fig. 19).

<Insert Figure 19>

With the intention of giving an application to these UPs, the authors used a DACC-PLLA film to attach a fluorescent dye (Fig. 20) by means of [2+2] cycloaddition reaction. After the photoreaction, the film was extensively washed to remove the unreacted dye and then analyzed by confocal fluorescence scanning microscopy. The results confirmed the fluorescent features of these new polymers. Based on the obtained results, the authors suggested that these polymers could be very useful to conjugate fluorescent targeting molecules.

<Insert Figure 20>

In a different, but very interesting approach, Kim and co-workers reported the synthesis and photocuring study of UPs in the presence of multifunctional thiols [90]. This experiment was conducted on the grounds that thiols can react with the double bonds through an anti-Markovnikov mechanism. The UPs were prepared from the polycondensation of FA with DEG and EG, and their end-groups were induced to react with trimethylolpropane diallyl ether in order to obtain an UP with allyl chain ends. For the photocuring reaction, the UPs were mixed with multifunctional thiols (with the ratio thiols/double bonds equal to 1), and the photoinitiator Irgacure 184. The reaction was monitored by FTIR and the results showed that the bands corresponding to the vinyl and thiol groups disappeared nearly at the same rate, suggesting a negligible homopolymerization of the UP through its double bonds. Using model compounds the

authors proved that the reaction of the terminal allyl double bonds with thiyl radical was faster than with the internal double bond. It was also found that the addition of aluminum tris(N-nitroso-N-phenyl-hydroxylamine) to the formulation contributed to increase its storage stability. The use of the multifunctional thiol [pentaerythritol tetrakis(3-mercaptopropionate)] led to the production of films with increased crosslinking density. This fact that was attributed to the high chain transfer ability of the thiyl radical.

The properties of UPs can also be tailored through the manipulation of their double bonds. One of the strategies to accomplish such modifications is based on the so called *click-chemistry*. In this context, Ates and co-workers [21] proposed the functionalization of polyesters through the use of thiol-ene click reactions on UPs that were obtained from the ROP of unsaturated macrolactone globalide (Gl), derived from non-toxic fatty acids. The ROP of globalide was promoted by the use CALB and then polyglobalide (PGl) was functionalized with compounds, *viz.* 6-mercapto-1-hexanol (MH), butyl-3-mercapto propionate (BMP) and N-acetylcysteamine (nACA) (Fig. 21).

<Insert Figure 21>

The different moieties used were able to introduce diverse chemical groups in the side chain of the UP. The use of MH led to the obtainment of an UP with –OH pendent groups. In the case of BMP and nACA, the authors foresaw the possibility of obtaining UPs with –COOH or –NH$_2$ groups, respectively. Additionally, the authors believe that this approach could be very useful in preparing a vast range of polymeric materials with potential application in the biomedical field.

Chanda and Ramakrishnan [20], demonstrated that it is possible to modify the double bonds of the UPs bearing itaconate moieties using Michael addition reactions. . Different UPs were prepared via bulk transesterification of dibutyl itaconate with different diols, *viz.* 1,12- dodecanediol, 1,20-icosanediol, 1,4 -cyclohexane dimethanol (CDM) and PEG, in the presence of dibutyltin dilaurate. The ^{1}H NMR analysis of the obtained UPs showed that the double bonds remained intact after the reaction, enabling a further functionalization, with different thiols and amines (Fig. 22). The ^{1}H NMR analysis of the products suggested a quantitative functionalization.

<Insert Figure 22>

Another type of modification that has been used is related with the epoxidation of the UPs double bonds, as already seen in Section 2.3. Roumanet and co-workers [91] synthesized oleic acid based UPs via bulk polycondensation of 1,18-(Z)-octadec-9-enedioic acid (D18:1) with different glycols (1,3-propanediol, 1,8-octanediol, 1,9-nonanediol, 1,10-decanediol, 1,12-dodecanediol). UPs with molecular weights ranging from 7000 to 20000 g/mol were obtained, with T_m ranging from 21 ºC to 45 ºC, being the lowest and highest value registered for the UPs with 1,6-hexanediol and 1,12-dodecanediol in the structure, respectively. The double bonds of the UP were then modified with epoxy groups through the reaction of the UP with m-chloroperbenzoic acid (mCPBA). The crosslinking reaction was then performed (by the formation of ether bridges), using UV light (photocrosslinking) and an iodonium salt as the photoinitiator. Transparent and highly cured films (< 5% of extractables) were obtained. The films were subjected to enzymatic degradation (pH=7.4, 37 ºC) in the presence of *Rhizopus Arrhizus* lipase. Results showed no significant degradation after 8 weeks which could be attributed to the highly hydrophobic nature of the films.

Kolb and Meier reported some interesting data related to the modification of saturated fatty acid methyl esters (FAMEs) [92] and proposed new methodologies for the modification of polyesters bearing pendant double bonds (Fig. 23) [93] *via* cross-metathesis with acrylates or *via* thiol-ene coupling modifications. The results proved that the methods used were efficient to introduce new functional groups on the structure of the polyester that could be used to tune their thermophysical properties.

<Insert Figure 23>

Regarding the development of partially biobased UPRs, some contributions are also presented in the literature. Olson and co-workers [94] prepared unsaturated poly(ester ether)s, using the methodology previously presented by Olson and Shears [12], and used the ensuing polymers in the preparation of biodegradable elastomers. The unsaturated polymers were thermally crosslinked in the presence of BPO and *N*-vinylpyrrolidone (NVP) as UM, to yield elastomeric films. Additionally, the materials were also fabricated into more complex forms with micropatterned surfaces making use of the lithography techniques. Overall, the results indicated that, depending on their composition, the crosslinked structures can be obtained with different *in vitro*

degradation profiles (from 30 days to 6 months) and also with a vast range of mechanical properties (Young modulus' ranged from 0.2 to 20 MPa).

In another contribution, Tang and Takasu [95] developed UPs bearing itaconate and maleate moieties through bulk polycondensation of maleic anhydride and itaconic anhydride with 3-methyl-1,5-pentanediol, in the presence of bis(nonafluorobutanesulfonyl)imide, under mild conditions (60ºC, 12 h, vacuum). The preparation of UPRs from the UPs encompassed two steps. First, a diamine was added to a mixture containing the UP, methyl methacrylate (MMA) and a UV-initiator. By means of Michael addition reaction, in particular through the reaction of the maleate units in the UP with the diamine, it was possible to obtain a single gel network (SN). Interestingly, it was found that the itaconate moieties did not participate in this reaction. Next, the mixture was subjected to UV irradiation, and the reaction of the itaconate moieties with MMA led to the formation of the UPR (DN gel) (Fig. 24). It was found that the ensuing UPR had high mechanical strength and exhibited shape memory properties. The authors suggested that this material could be useful in different areas but did not reference any specific application.

<Insert Figure 24>

Jasinska and Koning [96] prepared UPs from IS, SA and MA via thermal polycondensation in the presence of titanium(IV) n-butoxide [79]. The UPs showed a slightly branched structure that could result from the reaction of IS hydroxyl groups with the double bonds. The UPs, however, showed good thermal stability ($T_{5\%}$ above 260 ºC) and high T_g values (52 to 76 ºC). The UPs were further crosslinked in the presence of 2-butanone peroxide as initiator and cobalt (II) 2-ethylhexanoate as accelerator; styrene was replaced for less toxic monomers, *viz.* 2-hydroxyethyl methacrylate (HEMA), NVP, methacrylamide and AcA. UPRs with high T_g (60 to 103 ºC) were obtained and it was found that those were thermally stable up to 200ºC.

In another work, UPs with 'high green content', making use of different biobased monomers (e.g., IA, FA, IS, SebA) were prepared by Fonseca and co-workers [17] and their crosslinking reactions with HEMA studied. The UPs were prepared through bulk polycondensation in the absence of catalysts. The cure reaction was carried out at 80 ºC, using BPO as initiator. It was found that when IA was used as the unsaturated diacid, UPs with branched structures were obtained, as inferred by the high values of Đ (>4).

The UPRs presented high gel contents (>70 %), being the highest values obtained for the formulations with higher amounts of HEMA. The DMTA showed the existence of two transitions, indicating the presence of different domains within the UPR structure. Swelling tests were also carried out, and it was found that 20% was the maximum value attained, indicating that the UPRs have a highly hydrophobic nature. Additionally, the *in vitro* degradation tests (pH=7.4, 37 ºC) showed that the UPRs are hardly degraded under the tested conditions. The maximum weight loss was 9%, after 50 days.

In the last years, the use of vegetable oils, or their derivatives, in the preparation of new UPs and UPRs has gained significant interest in the scientific community. The oils were used both as monomers in the preparation of the UPs [72, 73, 97, 98] or in blends with commercial UPRs to increase their biobased content [74, 99]. To be used as monomers in the UPs synthesis', the vegetable oils primarily undergo an alcoholysis reaction (Fig. 25) with glycerol [72, 73, 98, 97], to yield a monoglyceride that is subsequently used in the polycondensation reactions.

<Insert Figure 25>

In a study by Mahmoud and co-workers [73], palm oil (PO) based UPRs were prepared and characterized. The PO based monoglyceride was made to react with MA, in the presence of 2-methylimidazole. The ensuing UP was then mixed with styrene (30 %wt) and UV-crosslinked, using Irgacure 184 as the photoinitiator. The results showed that the UPRs prepared from the UPs with 3 eq. of MA relatively to the monoglyceride showed the highest value of gel content and the best mechanical performance (high values of tensile strength and toughness).

In another interesting contribution, UPs made from glycerol, 1,4-butanediol and IA were prepared and were mixed with acrylated epoxidized soybean oil and water, in order to obtain stable waterborne dispersions [100]. The dispersions were then photocrosslinked in the presence of Irgacure 2959 and triethanolamine, as the initiator and accelerator, respectively. The authors tested the potential of the material to be used as a coating and the overall results showed that the crosslinked UPs presented excellent adhesion, high flexibility, and improved solvent resistance.

2.5 UPs and UPRs directed to biomedical applications

The double bonds in the UPs' structure, as previously discussed, can be easily modified using different approaches. This particularity is very advantageous in the biomedical context because it enables the attachment of specific moieties that could enhance the properties of the final material. The fact that some UPs can be crosslinked in the absence of UMs is also very promising, since the use of hazardous UMs is avoided. Nevertheless, and as it will be described in this section, efforts have been made to produce UPRs with less toxic monomers or with macromers based on materials with recognized biocompatibility. This subsection aims to present the most relevant works related with the use of UPs and UPRs in biomedical applications (e.g., tissue engineering or drug delivery systems).

Kharas and co-workers reported the synthesis of fumarate-based UP to be used in bioresorbable bone cement composites, replacing poly(methyl methacrylate) (PMMA), which is currently used in clinical practice [101]. The UPs were prepared by polycondensation of diethyl fumarate and different diols, namely 1,2-propanediol (PD), (*S*)-(+)-1,2-propanediol (SPD), 2-methyl-1,3-propanediol (MPD) and 2,2-dimethyl-1,3-propanediol (DMD). Different catalysts, namely PTSA, $AlCl_3$, $TiCl_4$, $Ti(OC_4H_9)_4$, and $ZnCl_2$, were also tested. The results suggested the possibility of controlling the UP structure and the extent of the side reactions by choosing the proper catalyst. For instance, the polycondensation of the monomers in the presence of PTSA led to branched UPs due to the occurrence of side reactions (between the hydroxyl groups and the double bonds), whereas in the presence of metallic catalysts an UP with a more regular structure and with high amounts of double bonds was obtained. After the polycondensation, the UPs were mixed with NVP, BPO and inorganic filler, $CaSO_4.2H_2O$, and cured in order to obtain a composite able to be used as a bone replacement material. The results showed that the composite presenting higher compressive strength and modulus was the one obtained based on the UP synthesized from PD in the presence of $ZnCl_2$. This fact was ascribed to the higher content of double bonds of this UP, that led to a more crosslinked composite, and also to the unbranched nature of the glycol used. The *in vitro* degradation tests (pH=7.4, 37 ℃) revealed that both the UPs obtained from branched glycols and the unbranched UPs (based on the polycondensation in the presence of metallic catalysts) are less susceptible to hydrolysis. Regarding the composites, it was found that those are less susceptible to hydrolysis than the corresponding uncrosslinked UPs. Nevertheless, the mass loss

tendency observed, was the same both for composites and their uncrosslinked counterparts.

Recently, Śmiga-Matuszowicz and co-workers [19] also reported a work in the field of UPRs as bone substitutes. The authors prepared porous scaffolds from the crosslinking reaction of poly(3-allyloxy-1,2-propylene succinate) (PSAGE) with oligo(isosorbide maleate) (OMIS) and a small amount of MMA, in the presence of BPO and *N, N'*-dimethyl-*p*-toluidine, as the initiator and accelerator, respectively. The foaming system used comprised calcium carbonate and L-LA or pyruvic acid. The results showed that the maximum temperature observed during the crosslinking reaction was 43 °C, and the crosslinked porous structure was able to be formed within 15 minutes. It was found that the scaffolds exhibited mechanical properties (e.g., compressive modulus and strength) similar to the trabecular bone. The *in vitro* cytotoxicity tests carried out with human fetal osteoblasts (hFOB1.19) cell line revealed that, after 24 h, the percentage of viable cells was between 77 and 99 %. Upon incubation in a phosphate buffered saline solution (pH=7.4, 37 °C) it was found that the porous scaffolds lose 40% of their initial weight after 26 weeks of degradation. Additionally, the scanning electron microscopy (SEM) analysis revealed that, after 3 hours of incubation, a crystal layer resembling apatite was formed at the surface of the scaffold. Taking into account the obtained results, the authors suggested that these materials can be used as fillers for bone voids.

Guo and co-workers [102] developed new UPs *viz.* poly(fumaric acid-glycol-dodecanedioic acid) (P(FA-GLY-DDDA)) copolymers, poly(fumaric acid-glycol-brassylic acid) (P(FA-GLY-BA)) copolymers, poly(fumaric acid-glycol-tetradecanedioic acid) (P(FA-GLY-TA)) copolymers and poly(fumaric acid-glycol-pentadecanedioic acid) (P(FA-GLY-PA)) copolymers. These were prepared in high yields by melt polycondensation of the corresponding monomers. The UPs were subsequently mixed with MMA, a dimer acid, and with BPO-triethylamine system, and it was found that the ensuing formulation was able to gel at temperatures close to human body temperatures. In terms of application in the biomedical field, the gels obtained from P(FA-GLY-BA) have shown to be the most promising taking into account its degradation profile (pH=7.4, 37 °C), its lack of toxicity when implanted in mice, and the release profile of hydrophilic drugs. Additionally, it was also found that the gels obtained from this UP (with a specific molar composition of 1.75FA/2.20 GLY/0.25BA) when used to encapsulate adriamycin hydrochloride were able to enhance the anti-tumor efficacy of the drug, as revealed by the *in vivo* tests carried out

on mice bearing Sarcoma-180 tumor. In a subsequent work, Guo and co-workers [103] reported the use of poly(fumaric acid-glycol-sebacic acid) (P(FA-GLY-SA)) copolymers and poly(maleic anhydride-glycol-sebacic acid) (P(MA-GLY-SA)) for the same purpose. The gels of both UPs have shown to be biocompatible and suitable for applications related with the drug delivery for cancer therapy.

Doulabi and co-workers [8] developed poly(ethylene glycol-co-fumarate) (PEGF) macromers to be used in the preparation of hydrogels. The macromers were prepared from PEG (1000 g/mol and 4000 g/mol) and fumaric acid, at 50 ºC, in the presence of the system dicyclohexylcarbodiimide (DCC)/dimethylaminopyridine (DMAP) as the condensation promoter (Fig. 26). The crystallinity and T_m were shown to be higher for the macromers prepared from PEG with high molecular weight.

<Insert Figure 26>

The hydrogels were prepared in the presence of a crosslinking agent, NVP, using a water soluble redox system comprising ammonium persulphate/*N, N, N', N'*-tetramethylethylenediamine as initiator. It was found that the gelation time to produce the hydrogels decreased with the increase in the PEG molecular weight. The swelling capacity was also higher for the hydrogels prepared from the macromers with PEG of high molecular weight.

In a different approach, Wang and co-workers [104] prepared a set of self-crosslinkable and biodegradable UPs from poly(ε-caprolactone-*co*-fumarate) (PCLF), poly(ethylene glycol-*co*-fumarate) (PEGF) and their copolymer PEGF-*co*-PCLF. The PCLF and PEGF were prepared from the reaction of the PCL-diol or PEG with fumaryl chloride, in the presence of K_2CO_3, as the proton scavenger. The copolymer was prepared in the same way, making use of the PCLF and PEGF macrodiols previously obtained (Fig. 27).

<Insert Figure 27>

The prepared UPs were able to be thermally or photocrosslinked, without the addition of a crosslinking agent. The authors suggested that these UP could be useful in the preparation of diverse materials for biomedical applications.

PCLF was used by Sharifi and co-workers [105] in the development of an *in situ* forming drug delivery system for tamoxifen citrate (TC), an anti-cancer drug. The networks were prepared via photocrossliking using camphorquinone (CQ) and

dimethyl-*p*-toluidine as the initiator and accelerator, respectively, and NVP as the crosslinking agent. Increasing the NVP content up to 16 %wt, led to an increase in the gel yield and a decrease in the swelling capacity. Above this value, the increment of the NVP concentration in the formulation did not translate in an increase of the gel yield, remaining some of the monomer unreacted. The *in vitro* cytotoxicity tests carried out on MCF-7 and L929 cell lines showed that the PCLF based networks containing 10 %wt NVP had no adverse effects on the cells. However, when PCLF/10%NVP networks were loaded with TC, it was observed that 40-60% of the cells were killed after 72 h of incubation.

In another approach, Sharifi and co-workers [106] synthesized novel self-crosslinkable and biodegradable polymers, namely poly(hexamethylene carbonate-fumarate) (PHMCF) and poly(hexamethylene carbonate) diacrylate (PHMCA), and their amphiphilic copolymers with PEG, poly(ethylene glycol fumarate-co-hexamethylene carbonate-fumarate) (PEGF-co-PHMCF). The synthetic route for the obtainment of the polymers was similar to that proposed by Wang and co-workers [104], but, in this case, instead of K_2CO_3, propylene oxide was used as the proton scavenger. The developed materials were able to be photocrosslinked, without the addition of a crosslinker or accelerator, and in the presence of camphorquinone (CQ). The *in vitro* cytotoxicity tests showed that the materials did not evoke any cytotoxic effect. The optimization of the photocuring parameters of PHMCF (Fig. 28A) were reported by Mohtaram and co-workers [107]. The photocrosslinking reaction was studied in the presence of CQ (0.5 to 1.5 %wt) and also in the presence of CQ and an amine accelerator, *viz.* dimethylamino methyl ethylacrylate (DMAMEA) (0.5 to 1.5 %wt) (Fig. 28B). The PHMCF has shown to be able to be photocrosslinked with a high degree of conversion (ca. 75 %), in the presence of CQ (1 % wt) and without DMAMEA. The network obtained from this specific photocrosslinking reaction showed the lowest swelling ratio and a total absence of cytotoxicity.

<Insert Figure 28>

UPs based on itaconic acid (IA), and the corresponding diester (dimethyl itaconate, DMI), AA, SA, and different diols, *viz.* sorbitol (Sorb), TMP, 1,4-cyclohexanedimethanol (CHDM), PEG and 3-methyl-1,5-pentanediol (MPDO) were prepared by Barret and co-workers [108]. Thermal and enzymatic polycondensation (mediated CALB) were used as the polymerization methods with IA and DMI,

respectively. Both branched and linear UPs were prepared (Fig. 29); the first ones were prepared from the copolymerization of IA with AA and TMP and from the copolymerization of SA Sorb, whereas the second ones resulted from the copolycondensation of DMI with CHDM, PEG and MPDO. The UPs were then photocrosslinked using 2,2-diethoxyacetophenone as the photoinitiator, and poly(ethylene glycol) methyl ether methacrylate (475 g/mol), or 2-(methacryloyloxy)ethyl acetoacetate as the UM. It was shown that the thermomechanical properties can be easily tuned by the choice of the monomers, being the best results obtained for the network based on the UP synthesized from DMI, AA, and MPDO. The materials have also shown to have low cytotoxicity, making them good candidates to be used in biomedical applications. Moreover, the suitability of the UPs to be used in imprint lithographic techniques was also shown.

<Insert Figure 29>

In another approach, Brown and co-workers [109] reported the synthesis of aliphatic UPs with potential application in the biomedical field making use of a new dicarboxylic acids and anhydrides (Fig. 30A), obtained from the Diels-Alder reaction of fumaric acid and maleic anhydride with several dienes. This synthetic route allowed the obtainment of new monomers with diverse functionalities, *viz.* ether and amine, that were preserved in the UP structure, after the polycondensation reaction with 1,8-octanediol, in the presence of $Sn(Oct)_2$ (Fig. 30B). All the UPs were amorphous, with T_g ranging from -30 to -15 ºC. The UPs were liquid at room temperature, and were able to self-crosslink in the presence of α,α′-azoisobutyronitrile (AIBN), at 130 ºC (Fig. 30C), yielding degradable elastomeric materials. These structures were evaluated in terms of their *in vitro* cytotoxicity, and the results showed that only the elastomer prepared from the UP containing the anhydride III (Fig. 30A) evoked a cytotoxic response. The remaining elastomers have shown to be non-cytotoxic.

<Insert Figure 30>

2.6 UPs, UPRs and additive manufacturing towards scaffold-based tissue engineering

The previous sections of this manuscript described the different strategies available in the literature to afford UPs and UPRs with tunable properties (e.g., thermomechanical, biodegradability, etc) for biomedical applications. Despite their huge potential, the design and production of 3D scaffolds using these materials remains very limited due to the processing techniques employed. The introduction of AM in the field of TE opens the possibility to generate 3D matrices with complex shapes, reproducible internal/external architectures, interconnected network of pores and multiple materials. Knowing the potential of UPs and UPRs as materials for AM due to the possibility of fine tuning their properties as previously described, in the current section of this contribution, the rationale behind TE and AM processes is presented with special emphasis on SLA systems. In particular, the main SLA processes, principles and applications will be described, as well as the recent advances in micro- and multi-material systems for the production of TE scaffolds.

As reported in the literature, TE represents an emerging multidisciplinary scientific field where principles of biology, medicine, materials and engineering are combined with the aim of developing artificial bio-implants for the regeneration/repair of damaged tissues and organs. The most common strategy in TE (scaffold-based TE strategy) comprises the combined use of cells and scaffolds to promote and enhance the healing process of the native tissues (Fig. 31).

<Insert Figure 31>

Most of cell lines used in TE are anchorage dependent, which means they will need a support template. For this reason, scaffolds play a major role in TE acting as a temporary biomechanical support to accommodate cells and promote the guided regeneration of the neo tissue [110]. The capacity of scaffolds to retain and deliver cells and biological molecules, promote the adequate diffusion of cell nutrients, oxygen and vascularization are fundamental aspects inherent to the regeneration process of tissues [111-113]. Due to the multi-functionality of human tissues it becomes very complex, if not impossible, to define which are the parameters of an ideal scaffold, even for a single tissue [114]. The considerations are complex and include chemical, morphological, mechanical and biological aspects, as well their variation with time. Specifically, a

scaffold must satisfy many requirements such as biocompatibility, controlled biodegradability and bioresorbability, interconnecting pores with specific size and geometry, appropriate mechanical and mass transport properties, as well as adequate surface topography and chemistry to promote cell adhesion, proliferation and differentiation [111-113, 115-127].

The mechanical, chemical and biological behavior of scaffolds are determined both by the intrinsic properties of the biomaterials as well the manufacturing processes used for their fabrication. In this sense, different technologies have been developed with the aim of producing scaffolds with optimized properties for TE applications.

Currently, it is possible to identify and classify the existing technologies into two major groups: conventional and non-conventional technologies.

Since the early days of TE, conventional technologies have been used extensively and with relative success in the fabrication of scaffolds. These methodologies include salt leaching, lyophilisation, supercritical fluids, moulding [111, 128-131].

These processes are generally quite simple from an implementation point of view enabling the production of highly porous structures. Despite this simplicity, they do not allow for a perfect control over the pore size, geometry and spatial distribution, leading to the generation of 3D structures with limited or inexistent pore channel interconnectivity. Besides the abovementioned limitations of these so called non-conventional techniques, the use of toxic organic solvents and long fabrication times are other drawbacks that have to be considered. Therefore additive manufacturing techniques are gaining increasing importance in the fabrication of 3D structures with customised external shape and predefined internal morphology, allowing good control of pore size and distribution.

Non-conventional or AM technologies represent a group of techniques recently introduced in the medical field. Besides the high reproducibility and elevated capacity to quickly produce very complex 3D shapes, these techniques enable the fabrication of scaffolds with good control over pore size and distribution increasing the vascularisation and mass transport of oxygen and nutrients throughout the scaffold [132-134]. The rationale behind AM technologies is based on the use of two dimensional data (2D) obtained from the slicing process of 3D models created via CAD (Computer Aided Manufacturing) systems to physically reproduce 3D objects in a

layer-by-layer fashion [135]. Fig. 32 illustrates the main steps required for the production of TE scaffolds using AM techniques.

The first stage requires the generation of the 3D solid model to be produced. This model can either be obtained directly from 3D CAD software, imported from 3D scanners or from medical imaging data (computed tomography, magnetic resonance, ultra sounds, etc.) [136].

<Insert Figure 32>

The CAD model is then tessellated into an STL model. This operation consists on the approximation of the model surfaces through a mesh of triangular elements. Finally the STL model is mathematically sliced into layers of homogeneous thickness (SLI file) and sent for production in one of the available AM techniques. The main advantages of AM techniques rely on the capacity of the systems to physically reproduce very complex 3D objects with relatively high speed and employing a wide range of materials. Through the combination of AM techniques with imaging data it becomes possible to produce personalized structures with optimal internal/external geometries, which will enhance the biomechanical coupling between the implant and the native tissue at the site of the defect. Fig. 33 illustrates some customized implants produced via AM techniques.

<Insert Figure 33>

Some of these processes can operate at room temperature, with relatively low mechanical work hence enabling the production of 3D hydrogel-based constructs with embedded cells (Fig. 34).

<Insert Figure 34>

Depending on the application, AM techniques allow for the direct or indirect production of 3D scaffolds. The last, requires the generation of a lost mould where the biomaterial can be deposited and give shape to the scaffold (Fig. 35). The existing or under development AM techniques employed in TE comprise SLA processes, sintering processes, extrusion, inkjet printing.

<Insert Figure 35>

The SLA processes, initially proposed by Hull and Pomerantz, involve the selective solidification of photosensitive resins [146, 147]. Currently, SLA processes comprise two distinct irradiation methods, namely mask irradiation and direct irradiation (Fig. 36).

<Insert Figure 36>

In the first method, each layer of the polymer is solidified via exposure to UV radiation, generated by a lamp and transmitted through a mask with transparent areas corresponding to the section of the model to be fabricated. This strategy can also be designated as integrative fabrication method as each layer is produced in full at once [148]. In the second method, also called vector photo-fabrication, a laser is employed to polymerize, point by point each layer of the polymer hence defining the cross section of the 3D object. The direct irradiation system is composed of a vat containing a photosensitive polymer, a movable platform where the model is built, a laser irradiating UV light and a dynamic optical system to direct the laser along the polymeric layer (Fig. 37). After the construction of each layer, the platform lowers inside the vat containing the polymer promoting the deposition of a non-reticulated polymeric film upon which the next layer will be generated.

<Insert Figure 37>

The methods described above comprise polymerization mechanisms (solidification or cure of liquid resins) through the absorption of a single radiation photon. However, there are processes based on the use of femtosecond laser emitting IR, where the polymeric system is solidified through a reaction ignited by the absorption of two radiation photons. This process is designated two-photon polymerization and enables the curing process to occur inside the polymeric system with nanometric resolution.

Independently from the adopted SLA strategy, two main events occur during the curing reaction, namely gelation and vitrification.

During the curing process, an increase in the T_g of the material may be observed, as a consequence of the increase in the molecular weight and cross-linking density. Generally, it is expected that by increasing the extent of cure, molecular weight and cross-linking will increase, thus removing free chain ends and reducing polymer chain

motions [149]. Consequently, the specific volume should decrease with increasing the extent of cure [149], and it might be expected an increase in density and modulus of the material as the conversion increases [148, 150].The kinetics of the curing process is strongly affected by temperature, light intensity, and resin composition. As reported in the literature [148, 151-153], photoinitiated curing processes are characterized by high initiation rates. Accordingly, volume shrinkage is much slower than the rate of chemical reaction and the system cannot be in volume equilibrium. For this reason, a temporary excess of free-volume is generated, thus increasing the mobility of the reactive species in the system. This effect induces higher reaction rates and conversions. Hence, the higher the temperature, light intensity, or initiator concentration, the higher the rate of gel formation which leads to higher conversion [148].

A wide range of materials can be used to produce 3D structures via conventional SLA such as agarose, dextran, PEG, PVA, poly(2-hydroxyethyl methacrylate) (PHEMA), etc. In this case the photo initiators must possess low cytotoxicity, enabling the polymerization of polymeric suspensions containing cells.

Liu and Bhatia [154] have developed a mask irradiation process for the production of cell laden constructs with micrometric resolution. In this process, the polymeric solution containing the cells is deposited in the working platform using a syringe (Fig. 38).

<Insert Figure 38>

Chan and co-workers [155] have developed a method to promote cell alignment on three dimensional hydrogel-based constructs through the combination of micro-contact printing and SLA (Fig. 39). Fibronectin was initially modified with acrylate groups and then used to pattern glass coverslips with acryl-fibronectin via micro patterning technique. Generated patterns were transferred to hydrogels produced via SLA and cell alignment investigated using NIH/3T3 mouse embryonic fibroblasts.

<Insert Figure 39>

Microstereolithography (micro-SLA) has evolved from conventional SLA process and possesses the same operating principles. High resolution 3D objects are built by superimposition of layers, each being produced by a light-induced photopolymerization of a liquid resin [133, 146, 156].

Since 1993 different strategies have been adopted to improve both the vertical (along the building axis) and lateral (in-plane) resolution of the SLA process, thus resulting in the development of several machines that can be classified into three categories:

1) Scanning micro-SLA systems, which are based on a vector by vector tracing of every layer of the object with a light beam;

2) Integral micro-SLA processes, which are based on the projection of the image of the layer to be built on the surface of the resin with a high resolution;

3) Sub-micron micro-SLA systems, allowing the polymerization of the layers which compose the structure directly inside the reactive medium. The improvement of the resolution is basically related to the reduction of the light-material interaction [157][.

All micro-SLA machines are based on the use of a liquid resin that should possess specific characteristics, since it is directly related to the resolution of the object that will be manufactured. One important development in this area was the development of a novel integral SLA system by researchers from the Centre for Rapid and Sustainable Product Development (CDRSP), Portugal. This system comprises a light source consisting on a 200 Watts mercury lamp with 3 fundamental peaks of irradiation (320-390 nm with radiation intensity of 17 Watts/cm^2, 390-450 nm with radiation intensity of 20 Watts/cm^2 and 280-320 nm with radiation intensity of 7 Watts/cm^2), a *shutter* for the radiation on/off, a set of filters and collimating lenses which guarantee the adequate selection of the wave length, a UV radiation DMD (*Digital Micro-Mirror Device* DMD 0.7 XGA 12º DDR, 1024x768 pixel of 14 μm dimension) which enables the micro definition of each cross section to be built, a reduction Zeiss lens (10X maximum reduction) and building moveable platform with a resin vat (Fig. 40).

<Insert Figure 40>

This system allows for the production of hydrogel-based constructs for TE applications (Fig. 41). The structures are formed by physical or covalent reticulation of a liquid polymeric solution transforming it into a solid hydrogel.

<Insert Figure 41>

In the TE field, micro-SLA has been used, for example, to produce customized poly(propylene fumarate) (PPF)/diethyl fumarate (DEF) photopolymer 3D scaffolds

incorporating BMP-2 loaded PLGA microspheres [158]. In this case, PLGA microspheres were incorporated into a 3D scaffold which was fabricated through a micro-SLA system with a suspension of microspheres and a PPF/DEF photopolymer. The *in vitro* release profiles were assessed, and it was demonstrated that the produced microsphere-containing 3D scaffold was able to gradually release the growth factor. The biological performance of such multifunctional structures was analysed, confirming that scaffolds produced by micro-SLA were superior to traditional structures obtained using particulate leaching/gas foaming method.

However, as reported in the literature [159, 160], excimer-laser-based projection photocuring of liquid resins represents a promising method to increase the production efficiency of SLA, taking into consideration the well-shaped and high-power beams delivered by excimer lasers. In addition, such lasers may offer the possibility to process a wide range of materials for scaffold fabrication as the wavelengths can span all the deep-UV to UV range [159, 160].

For this reason, an excimer-laser-based SLA was also developed and proposed as a rapid process to fabricate PPF/DEF scaffolds for TE [160].

The results obtained from experimental analyses demonstrated that the proposed technique could be considered as an excellent tool for the development of biocompatible structures on a micrometre scale showing high-resolution and well-defined patterns in all the three spatial dimensions. Furthermore, the speed of the process was strongly increased, thus favoring a potential translation of this technology to clinical applications [160].

A scalable method to synthesize well-defined PPF was also reported by means of a ring opening polymerization of maleic anhydride and propylene oxide coupled with a post polymerization isomerization reaction [161]. The biological evaluation of these PPF oligomers was performed and their use to manufacture 3D printed scaffolds via established photochemical methods was assessed.

In particular, DEF was also added to the PPF in a specific mass ratio to reduce the viscosity of the polymer. The photoinitiators Irgacure 819 and Irgacure 784 together with oxybenzone and additional DEF were added to the PPF/DEF mixture to optimize the final composition of a suitable reactive solution for photo-cross-linking. Preliminary analyses showed that the 3D printed scaffolds were nontoxic to L929 mouse fibroblasts and human mesenchymal stem cells [161].

To manufacture multi-material functionally graded components, researchers from the CDRSP, Portugal, have developed a new SLA process, which is known as stereo-thermal-lithography (STLA) [148, 162]. This fabrication process uses UV radiation and thermal energy produced by IR radiation to initiate the polymerization in a medium which contains both photo- and thermal-initiators (Fig. 42) [148, 162].

<Insert Figure 42>

The concentrations of these initiators must be properly selected and the curing process starts when there is a specific combination of thermal and UV radiation energy [148, 162]. Also, the amount of each initiator must be low to prevent the start of the polymerization by only one of these two effects.

In this fabrication process, temperature is used to produce radicals through the thermal decomposition of the initiators, simultaneously increasing the initiation and reaction rate of the photoinitiated curing reaction. Consequently, the extent of cure increases and no post-cure should be needed, thus producing high quality models [148]. If compared to conventional SLA, STLA presents several advantages, such as: a more efficient generation of radicals; the use of small concentrations of two different types of initiator which enables the radiation to penetrate deeper into the material; a faster reaction rate; a fractional conversion values due to the specific combination of UV radiation and temperature; and an improvement of the accuracy of the produced models as a consequence of a more localized curing reaction, a more tunable system [148].

In particular, the overall system may be divided into four subsystems:

- ➢ Subsystem A employs UV radiation to solidify a liquid resin containing a specific amount of photoinitiator. It corresponds to an approach which is similar to conventional SLA.
- ➢ Subsystem B uses thermal energy produced by infrared radiation for solidifying a liquid resin which contains a specific amount of thermal initiator.
- ➢ Subsystem C employs both heat produced by infrared radiation and ultraviolet radiation in order to solidify a liquid resin which contains a certain amount of photoinitiator.
- ➢ Subsystem D uses both heat produced using infrared radiation and ultraviolet radiation to solidify a liquid resin containing a certain amount of thermal initiator and photoinitiator [148].

Furthermore, the system also contains a rotating multi vat allowing the production of multi-material structures (Fig. 43).

<Insert Figure 43>

This should represent an important advancement in developing multi-material functional graded scaffolds for tissue engineering, multi-material microscopic engineering prototypes through nanostructures for different applications in several fields, biomedical components, as well as functional metallic or ceramic parts [148].

In this context, our research group developed new bio UPs to be applied in SLA [18]. In particular, the different biobased UPs (Fig. 44) were used to prepare scaffolds by micro-STLA, using HEMA as UM and Irgacure 651 as photoinitiator.

<Insert Figure 44>

The obtained structures presented very accurate external geometries (Fig. 45), well defined internal pores and nano scale surface roughness, which is very important to promote cell adhesion and proliferation.

<Insert Figure 45>

Two-photon polymerization (TPP) may be considered as a useful SLA strategy to fabricate micro/nanoscale structures. Basically, femtosecond laser pulses are focused into the volume of a liquid resin transparent to the infrared radiation without using photomasks (Fig. 46) [148, 163-170].

<Insert Figure 46>

In this fabrication process, the reactive molecule should be able to simultaneously absorb two photons instead of one as it is excited to a higher singlet state. The process allows a submicron 3D resolution, also allowing 3D fabrication at greater depth and ultra-high speed [148].

With regard to the fabrication of 2D and 3D microstructures with TPP, a nanostereolithography (nano-SLA) system was also developed (Fig. 47) [148, 167].

<Insert Figure 47>

It involves the use of a mode-locked Ti:sapphire laser as the light source, providing a wavelength of 780 nm and a pulse width of less than 100 fs at a repetition rate of 80 MHz [107, 172]. The beam is scanned across the focal plane by employing a set of two Galvano-mirrors characterized by a resolution of approximately 2.5 nm per step, and along the vertical axis using a piezoelectric stage. The laser is closely focused into a volume of photocurable resin with an objective lens. In order to obtain exposure times less than 1 ms, a Galvano-shutter combined with a pin-holed plate is applied [167]. A control program is used to control the shutter, scanner, and piezoelectric stage. A high magnification CCD camera is also employed to monitor the fabrication process [167]. The contour scanning method and the raster scanning method have been considered to produce micro-objects with TPP. In the contour scanning method, the contour profile of the microstructure is traced by the laser beam, whilst in the raster scanning method all of the cubic volume containing the microstructure is scanned. The contour scanning method improves the fabrication efficiency, requiring less processing time [167]. In the nano-SLA process, according to the scanning paths one sliced layer of the 3D microstructure is solidified. After translating the position of the beam spot along the z-axis, another layer is produced. The 3D structure is sequentially produced with this method [167]. TPP using resins which contain conventional initiators have been reported in the literature, even if such polymeric systems exhibit low photosensitivity [148, 169, 173]. Commonly used materials are inorganic–organic hybrid materials whose properties can be suitably tailored benefiting from a combination of the characteristics of their constituents, polymers and inorganic glasses [174, 175].

Even though TPP possesses many advantages, this technology still has some problems related to the fabrication of multi-material structures [176].

To sum up, SLA processes present significant advantages for the production of TE scaffolds, namely high dimensional accuracy, capability to generate very complex geometries, possibility to use multiple materials and ability to operate at room temperature (or above) for the encapsulation of cells and biological molecules. Significant advances are expected in the next few years with the integration of different technologies into a single hybrid system capable of replicating the nano, micro and macro features of the tissues, introducing biomimetic functional gradients and improving the overall regeneration process.

2.7 Experimental design and theoretical concepts in SLA

To develop scaffolds for TE using SLA process, the materials must fulfill some requirements such as biocompatibility, fast curing process and low viscosity.

In the SLA process, the UV radiation, which hits the surface of the liquid resin, is absorbed and dispersed.

Accordingly, the penetration of the radiation is limited and, hence, the depth of curing which may be defined as follows [148, 178]:

$$C_d = D_p \ln\left[\frac{E_{max}}{E_c}\right] \qquad (1)$$

where, C_d represents the maximum curing (penetration) depth, D_d is the penetration depth at which the beam intensity is reduced to $1/e$ of its surface value, E_{max} is the maximum energy value on the resin surface and E_c is the critical energy value which is required for the liquid $\rightarrow$ solid phase transition of the resin. As a consequence, the depth of curing is a function of the exposure and may be controlled by varying the radiation level applied to the liquid resin. The maximum exposure energy on the resin surface may be written as follows [148, 178]:

$$E_{max} = \sqrt{\frac{2}{\pi}} \frac{P}{w_0 v_s} \qquad (2)$$

where P represents the laser power within the vat of the stereolithography apparatus, w_0 and v_s are the beam radius and the scanning speed, respectively [148].

An insufficient extent of cure means that it is necessary to perform a post-cure operation in an ultraviolet chamber [148].

Accordingly, the knowledge of the material and process parameters is crucial to further extend the use of SLA processes and to determine the suitable resin formulations.

The evaluation of the molecular mass and molecular mass distribution as well as of the calorimetric properties of the material (*e.g.*, glass transition, thermal degradation…) represents an important step to optimize the SLA process.

The curing behavior can be properly optimized by analyzing the curing depth as a function of the irradiation dose or time. The effect of the light intensity on the curing process can be properly assessed, fractional conversion as a function of curing time provides an important information for the process optimization.

All of this may allow to tailor the reaction rate, as well as the induction time and vitrification.

At different molecular mass levels, to assess the flow behavior measurements of intrinsic viscosity may be performed by a capillary viscosimeter, whereas steady shear tests may allow to analyze the viscosity as a function of the shear rate.

On the other hand, small amplitude oscillatory shear tests can be also carried out to evaluate the viscoelastic properties of the resin (*e.g.*, storage modulus and loss modulus as a function of frequency).

In many cases, the evaluation of the viscosity may be difficult as a possible consequence of the high concentration of carbon-carbon bonds in the polymer backbone and eventual crosslinking reactions which can occur during the polymerization reaction [18].

Furthermore, rheological stability or instability may be evaluated by monitoring the viscosity as a function of the shear rate over time.

It is well known that high molecular mass polymers with high viscosity are usually not suitable for manufacturing 3D printed scaffolds [161].

For this reason, taking into account the above reported features, it is possible to define a scalable method to synthesize a material with well-defined molecular mass, narrow molecular mass distribution and viscosity properties suitable to manufacture 3D scaffolds using SLA processes.

As reported in the previous sections, our group focused on the development of different biobased UPs to be used in micro-SLA [18], trying to overcome the lack of proper biocompatible and photocurable polymers [18, 178].

Specifically, the viscosity values of UPs based on alkyl carboxylic acids were low (*e.g.*, 0.24 -0.58 Pa s) in comparison with those based on isophthalic acid. This difference can be due to the higher mobility of the polymer chains incorporating a long alkyl chain backbone [18]. An extensive characterization showed that the properties of these UPs matched the micro-SLA requirements, thus opening the possibility to develop new biodegradable and tailor made scaffolds for TE [18].

3. Conclusions and Outlook

The introduction of AM technologies in the TE field brought an unprecedented toolbox of methods to produce tailor made 3D structures. In order to explore the full potential of

these advanced methods it is crucial the development of suitable polymers that can fulfill some demanding requirements.

There are still many barriers to use innovative materials and advanced SLA processes in the mainstream clinical practice. For example, one of the main problems is related to the synthesis of low molecular weight polymers with reproducible properties and well-defined characteristics that could be used in SLA. Recently, the development of new UP based materials have received enormous interest for biomedical applications, particularly in the development of tailor made 3D structures. These polymers can be successfully used in AM due to several important features: biocompatibility, easy synthesis, functional side groups, large availability and fast UV/Vis curing. Although important developments have been made over the last years regarding the synthesis of UPs, their full use in AM requires important research efforts to overcome some existing issues that strongly affect the manufacturing process. Among them it is possible to stress: the rheological instability that leads to an increase of viscosity over time; non-reversible events; and phase transitions. The rigorous control over the structure of the polymer is also mandatory for the fine adjustment of the mechanical properties of the final 3D scaffolds aiming specific applications, the optimization of cell attachment, growth and proliferation. In this vein, it is frequently reported that poorly controlled manual cell seeding should be overcome by AM technologies that may allow the fabrication of cell-containing constructs in a computer controlled manner. However, most of the limitations are related to the discussed lack of polymers with appropriate properties and processability.

Regarding the SLA technologies, the developments achieved during the last decade are remarkable. The accuracy of most SLA systems is considerably higher compared to other AM techniques enabling the production of TE constructs with sub-micron resolutions. The flexibility of SLA, especially in terms of the light sources used, combined with the freedom of design that it offers, results in the ability to create highly detailed functional implants. However, the successful application of these light-based systems in TE is still hindered by some technological issues: 1) long printing time: this issue becomes especially relevant for the design and production of cell-laden constructs. The majority of SLA systems are still very slow hence affecting cell viability and proliferation after encapsulation; 2) multi-material printing: currently there are two approaches to produce multi-material scaffolds using SLA. The first implies the

exchange of the entire material vat during fabrication and the second requires the exchange of the materials inside the single vat. Despite being possible to print multi-material 3D constructs, more efforts will be needed to eliminate problems associated with cross contamination between different resins, uncured material that affects layer accuracy and layer interfaces. The last is particularly important for the design of 3D constructs with continuous functional gradients and improved mechanical stability; 3) gravitational settling of cells: if the printing speed is too low and the resin does not contain the adequate rheological properties, cells will not be homogenously distributed within the precursor solution. Thus, modifications in terms of the SLA apparatus (i.e. introducing centrifugational forces) and in the resins (i.e. including self-adhesive peptides in the cell solution) will be required to improve the design of cell-laden implants; 4) convergence of techniques: the integration of SLA with other AM techniques such as dispensing systems would enable, for example, to combine high accuracy (from SLA) with cell friendly processing (from dispensing) for the production of accurate and biologically viable 3D cell laden-constructs.

To sum up, the synthesis of novel materials and the ways to tune their performances together with the more advanced SLA processes (from micro-SLA and STLA to TPP and nano-SLA) will allow the fabrication of 3D constructs with tailored mechanical, chemical and biological functionality to improve cell-cell and cell-matrix interactions hence leading to a better understanding of the complex cellular mechanisms involved in the regeneration of tissues and organs. With a more synergetic approach between materials scientists, engineers and clinicians it will be possible to design and produce novel biomaterials for SLA opening avenues for effective solutions that can be translated to clinical practice.

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(A)

Propylene glycol   Isophthalic acid   Maleic anhydride

200-205 °C

(B)

Styrene

Δ
or
h.υ

Initiator
Accelator
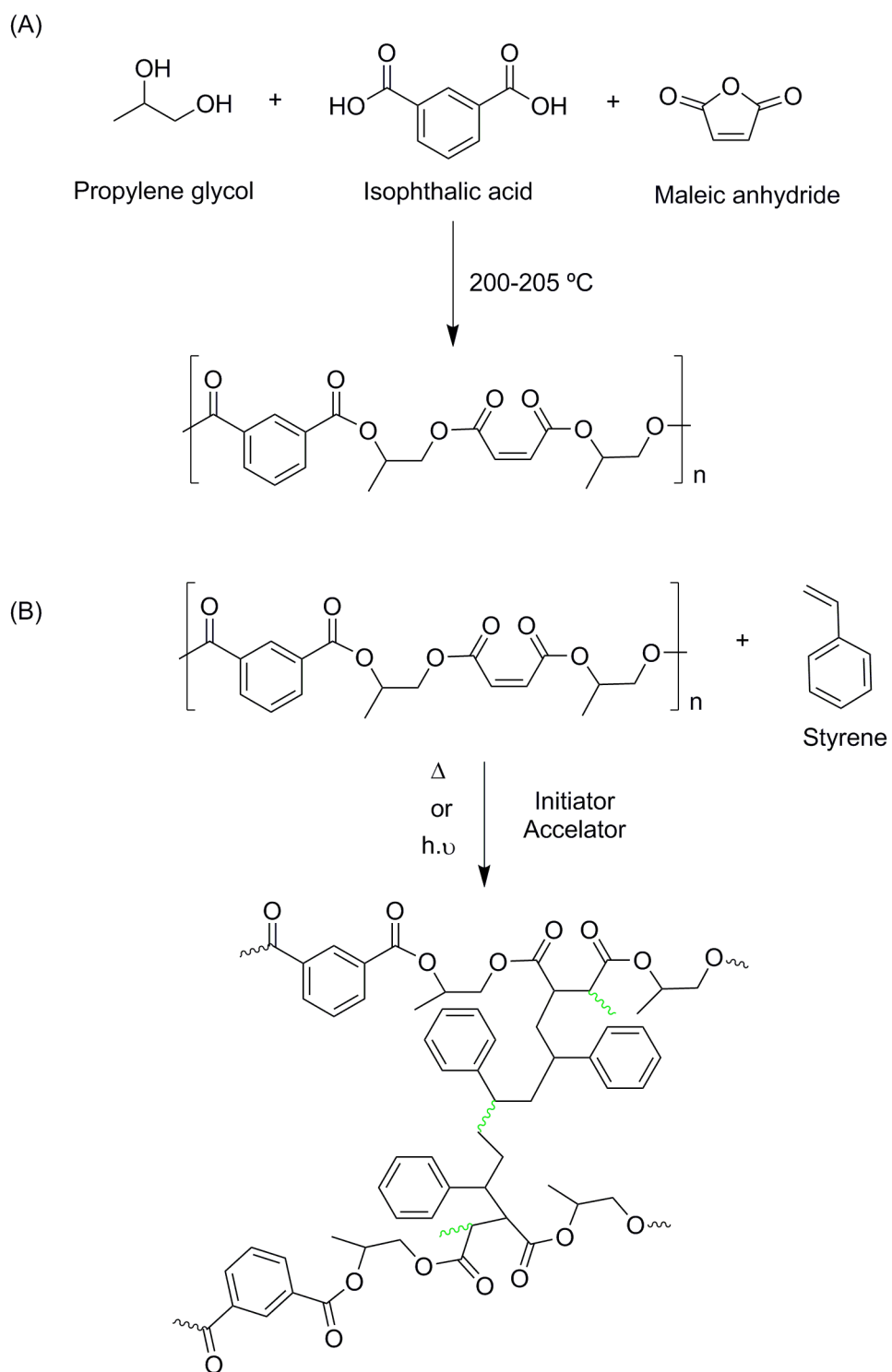
Fig. 1. Path for the development of UPRs: (A) Polycondensation reaction, and (B) cure reaction.
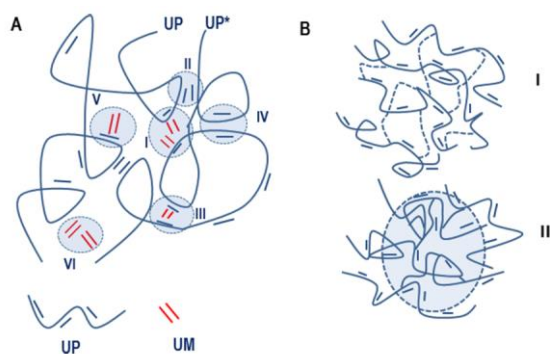
Fig. 2. A) Schematic representation of the possible reactions in the UM-UP cure reaction: (I) intermolecular crosslinking UP-UM-UP*; (II) intermolecular crosslinking UP-UP*; (III) intramolecular crosslinking UP-UM-UP (or UP*-UM-UP*); (IV) intramolecular crosslinking UP-UP (or UP*-UP*); (V) branching growth UP-UM (or UP*-UM); (VI) UM homopolymerization. B) Formation of microgel particles through the growth of free radicals: (I) growth of free radicals; (II) formation of microgel particles. [25], Copyright 1988. Adapted with permission from Elsevier Ltd.

(A)

(B)
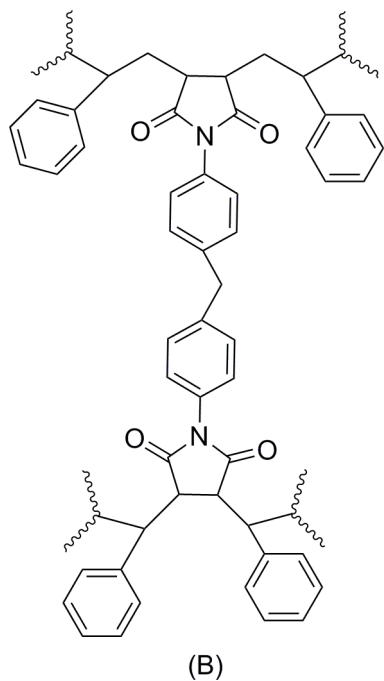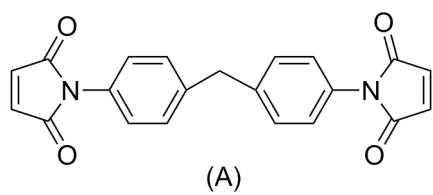
Fig. 3. Structures of : (A) BM, and (B) BM-styrene bridges [42].

(A)
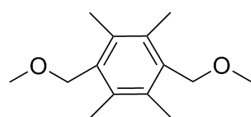
3,6-bis(methoxymethyl)durene
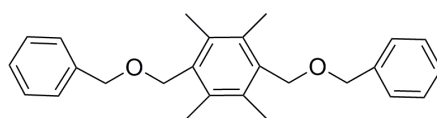
3,6-bis(benzyloxymethyl)durene

(B)
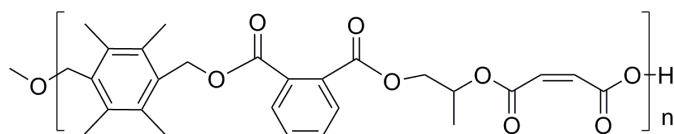
I
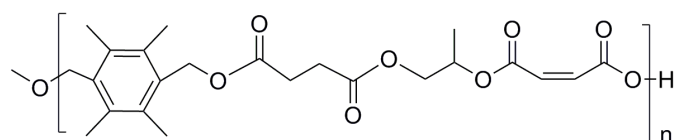
II
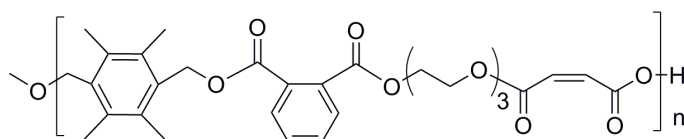
III
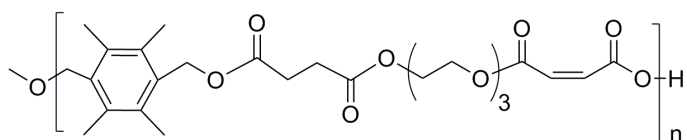
IV
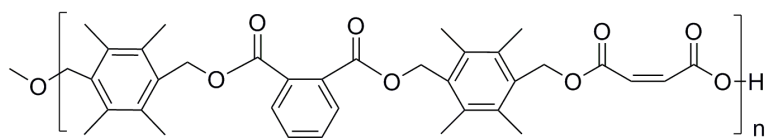
V

Fig. 4.   Monomers used for the preparation of durene based polyesters (A), and structure of the UPs (B) [47].
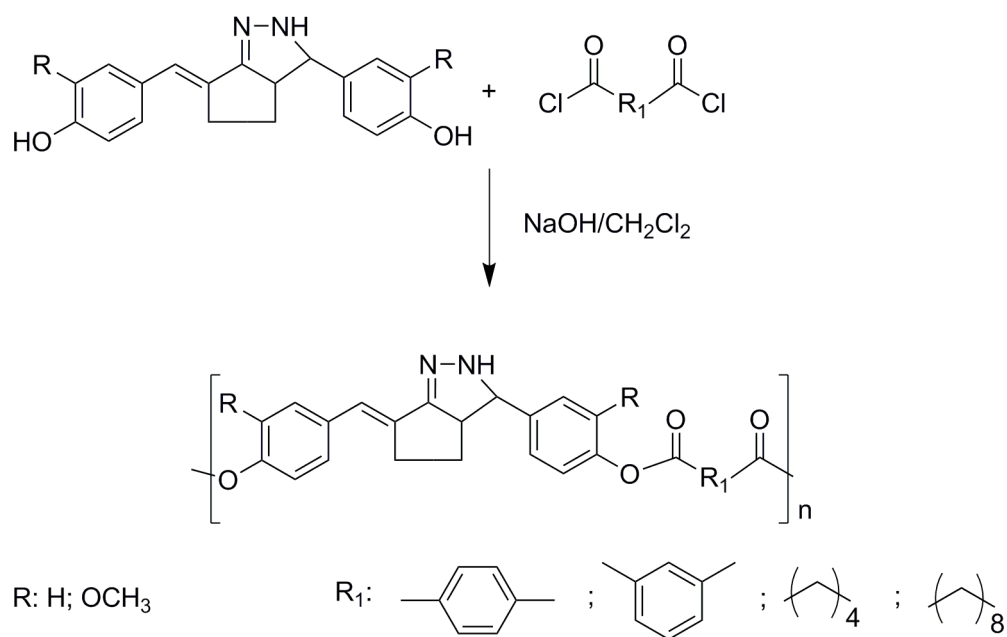
Fig. 5.   UPs synthesis through interfacial polycondensation technique. [49],

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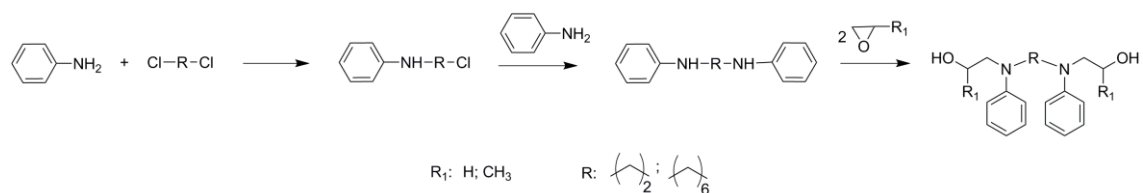
Fig. 6. Scheme of preparation in two steps of amine glycol modifiers for UP resin, i.e., 3,6-diaza-3,6-diphenyloctane-1,8-diol (R=-$(CH_2)_2$-; R_1=-H), 4,7-diaza-4,7-diphenyldecane-2,9-diol (R=-$(CH_2)_2$-; R_1=-CH_3) and 3,10-diaza-3,10-diphenyldodecane-1,12-diol (R=-$(CH_2)_6$-; R1=-H). [56], Copyright 2001. Reproduced with permission from John Wiley & Sons Inc.
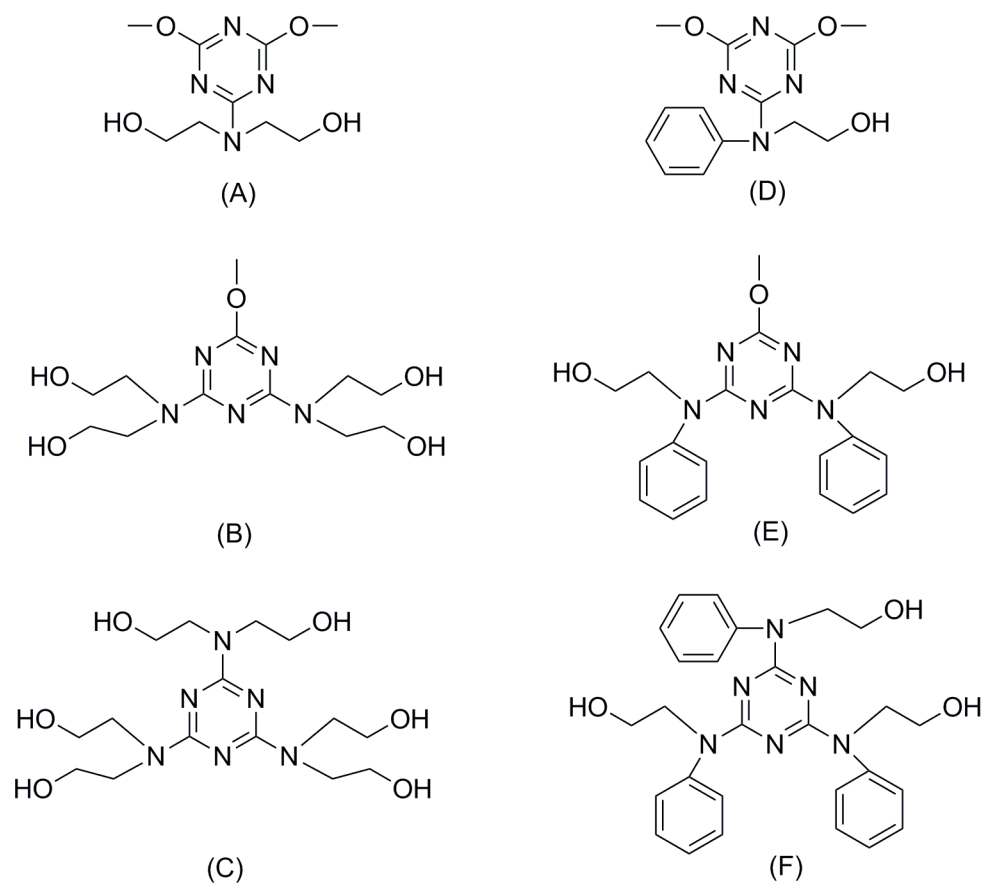
Fig. 7. Structures of the amine diols and polyols bearing *s*-triazine rings: (A) 2-[N,N-bis(2-hydroxyethyl)amine]-4,6-dimethoxy-1,3,5-triazine; (B) 2,4-[N,N-bis(2-hydroxyethyl)amine]-6-methoxy-1,3,5-triazine; (C) 2,4,6-tris[N,N-bis(2-hydroxyethyl)amine]-1,3,5-triazine; (D) 2-[N-phenyl-(2-hydroxyethyl)amine-4,6-dimethoxy-1,3,5-triazine; (E) 2,4-bis[N-phenyl-(2-hydroxyethyl)amine]-6-methoxy-1,3,5-triazine; and (F) 2,4,6-tris[N-phenyl-(2-hydroxyethyl)amine]-1,3,5-triazine [57, 58].
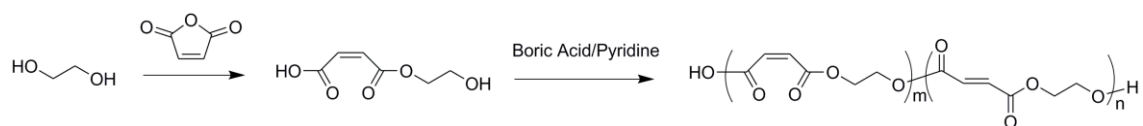
Fig. 8. Polyesterification of the monohydroxyethyl esters of maleic acids with boric acid-pyridine mixture as mild catalyst. [60], Copyright 2010. Adapted with permission from Elsevier Ltd.
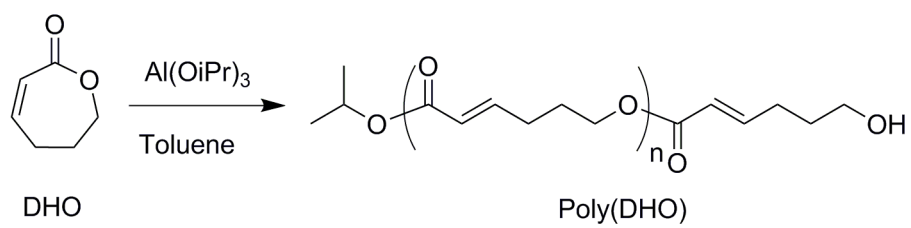
Fig. 9. ROP of DHO to give the poly(DHO) [61].

R: -CH₃; -CH₂CH₂OCH₃; -CH₂CH₃
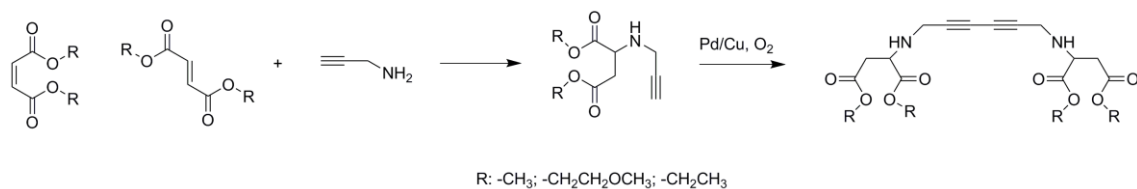
Fig. 10. Metal-mediated reaction of UP via functionalization of maleic or fumaric diesters, followed by oxidative homo-coupling of the introduced alkyne moieties [64].
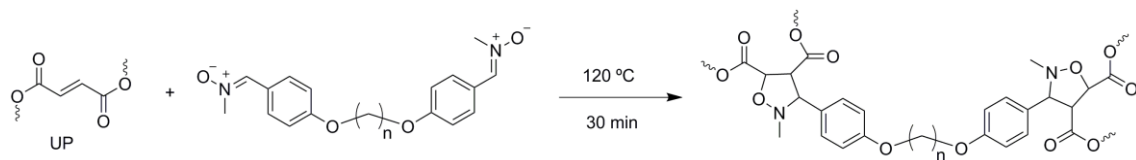
Fig. 11. Crosslinking reaction between the UP and a polynitrone, leading to a 3D network [65].

(A)

(B)
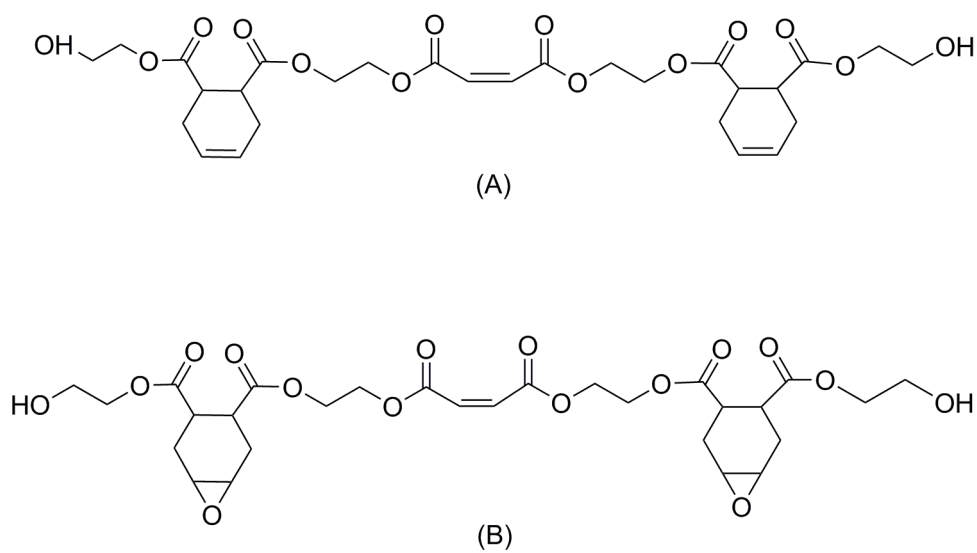
Fig. 12. Structures of: (A) unsaturated oligoester; and (B) unsaturated epoxyoligoester, using EG as the diol.
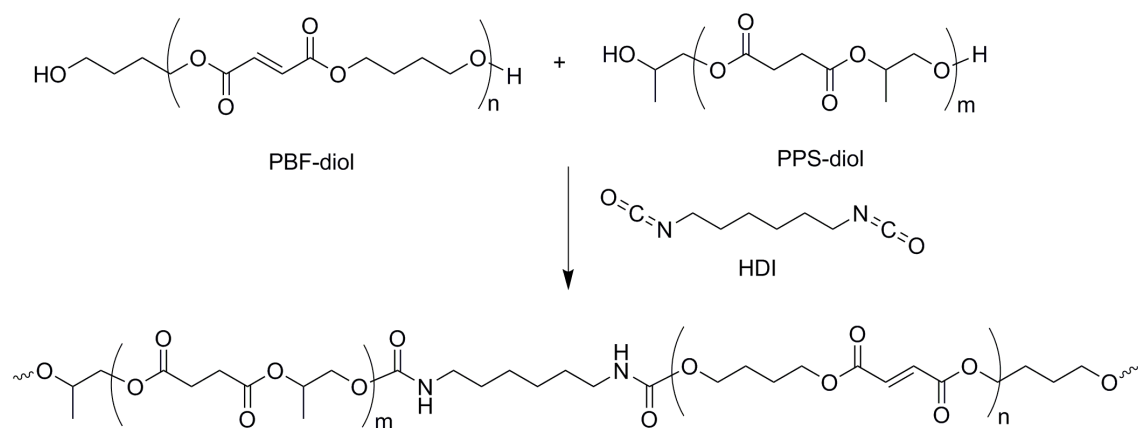
Fig. 13. Chain extension reaction of PPS-diol and PBF-diol mediated by HDI.
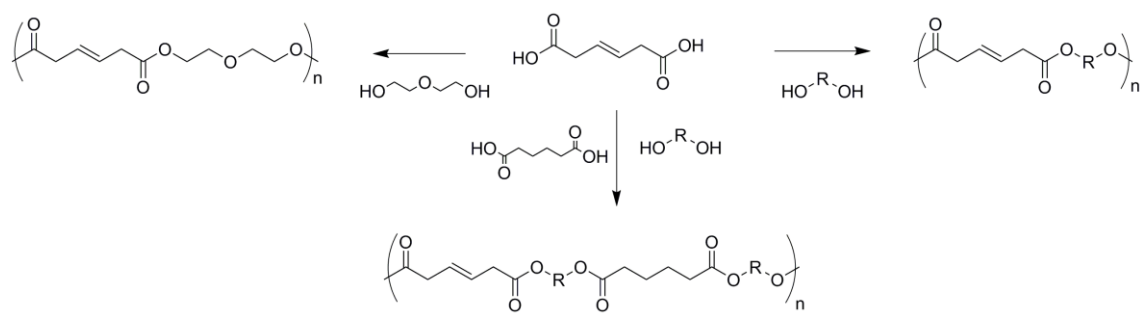
Fig. 14. Synthesis of HMA homopolymers, HMA-adipic acid copolymers and of HMA and diethylene glycol poly(ester ether)s [12].

Diethyl adipate

Diethyl fumarate

Diethyl itaconate

Diethyl *trans*-glutaconate

Diethyl *trans*-β-hydromuconate
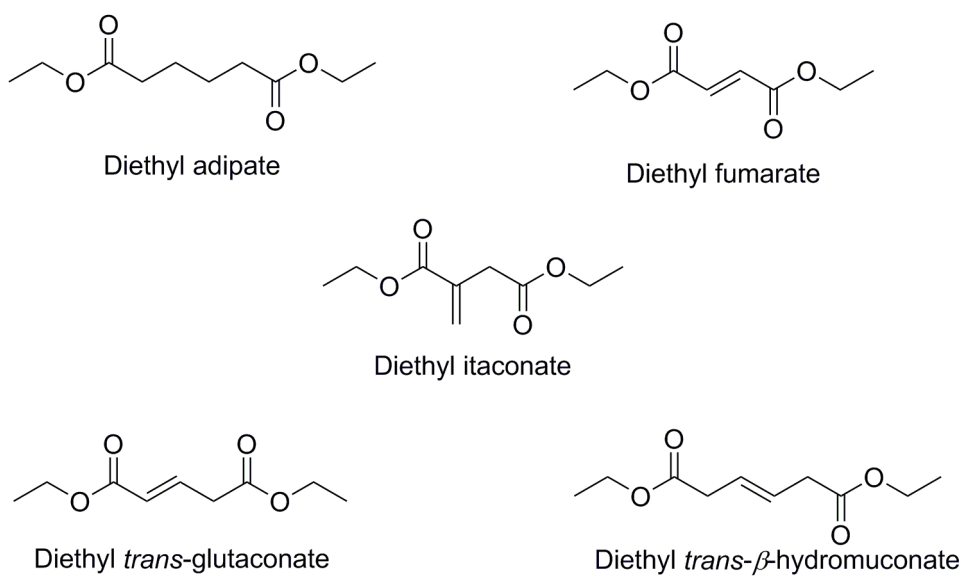
Fig. 15. Structures of the synthesized diesters, diethyl adipate, diethyl fumarate, diethyl itaconate, diethyl *trans*-glutaconate and diethyl *trans*-β-hydromuconate used for further transesterification reaction with isosorbide or isomannide.

Diethyl succinate   Itaconate   1,4-butanediol

Solution Polycondensation | 1) Oligomerization, 80 ºC, 2-24h, N₂
CALB | 2) Polycondensation, 80 ºC, high vaccum
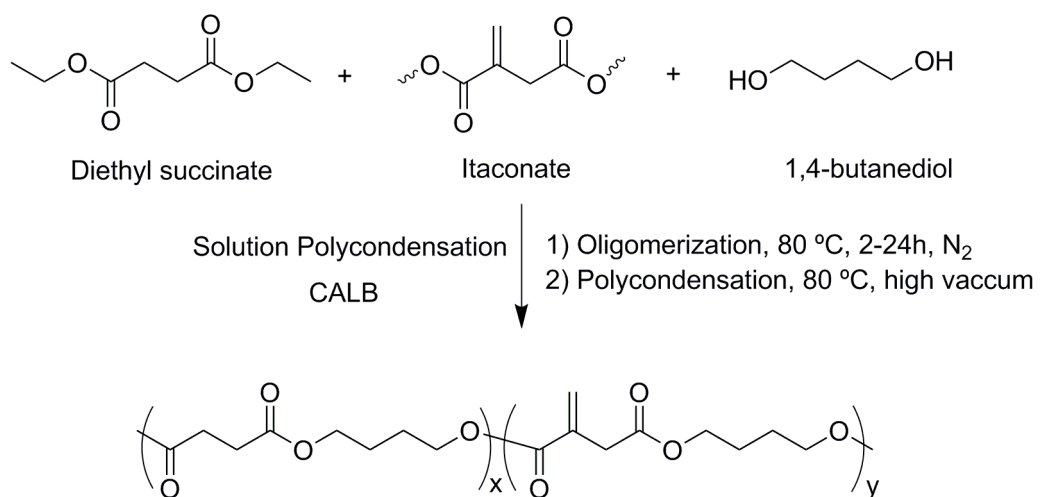
Fig. 16.   Preparation of the UPs by the two-step solution polycondensation [70].

(A)   (B)
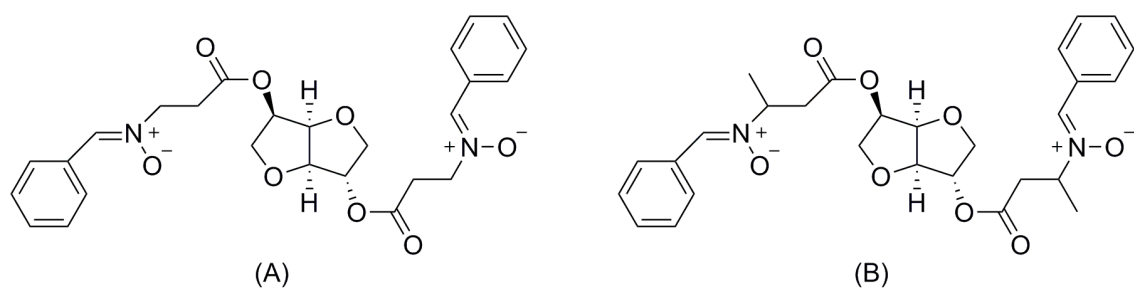
Fig. 17.   Structures of the *N*-alkylated dinitrones based on IS [76].
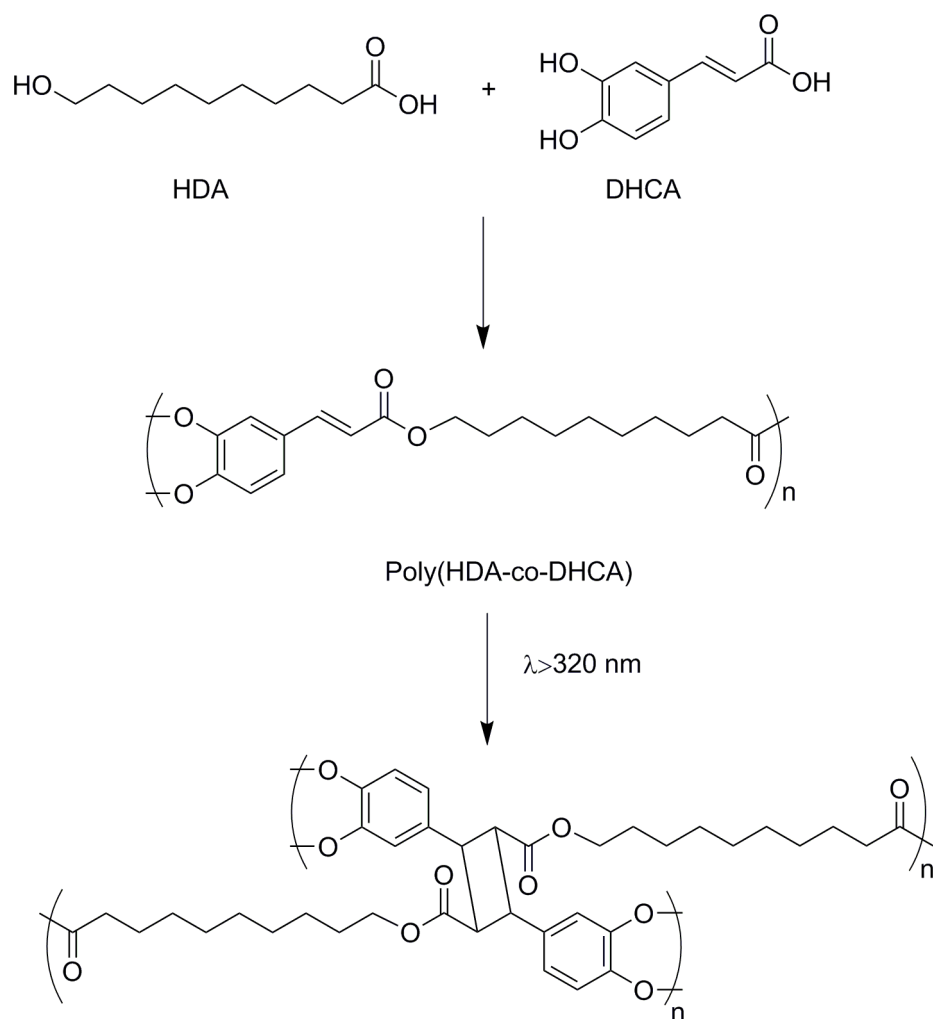
Fig. 18. Scheme of the synthesis of poly (DHCA-HDA) and photoreaction of the DHCA moiety promoted by UV irradiation [86].
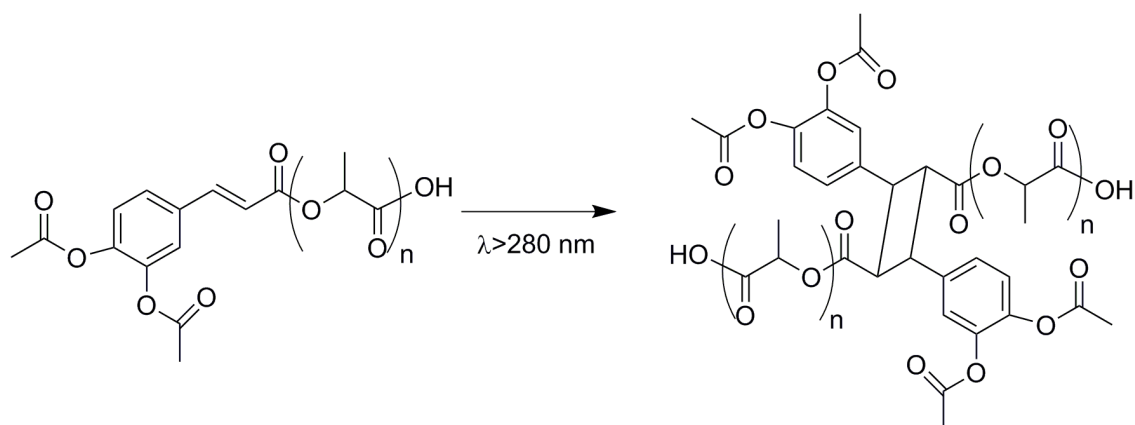
Fig. 19. Photoreaction scheme of DACA-PLLA by UV irradiation at λ>280 nm. [88], Copyright 2008. Reproduced with permission from the Royal Chemistry Society.
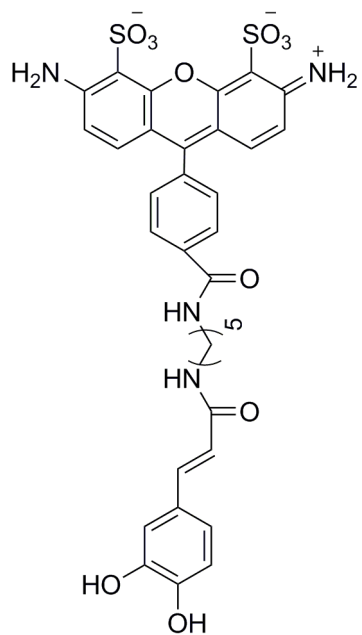
Fig. 20. Structure of the fluorescent dye.
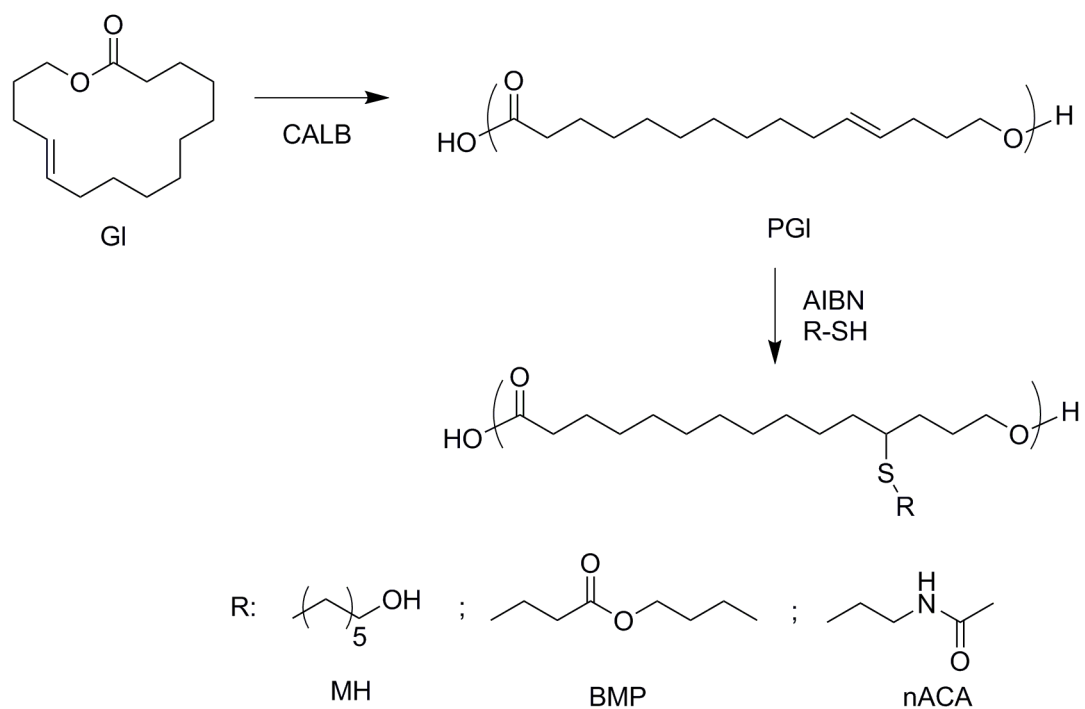
Fig. 21. ROP of globalide and thiol-ene reaction of PGl with MH, BMP and nACA. [21], Copyright 2011. Reproduced with permission from the Royal Chemistry Society.
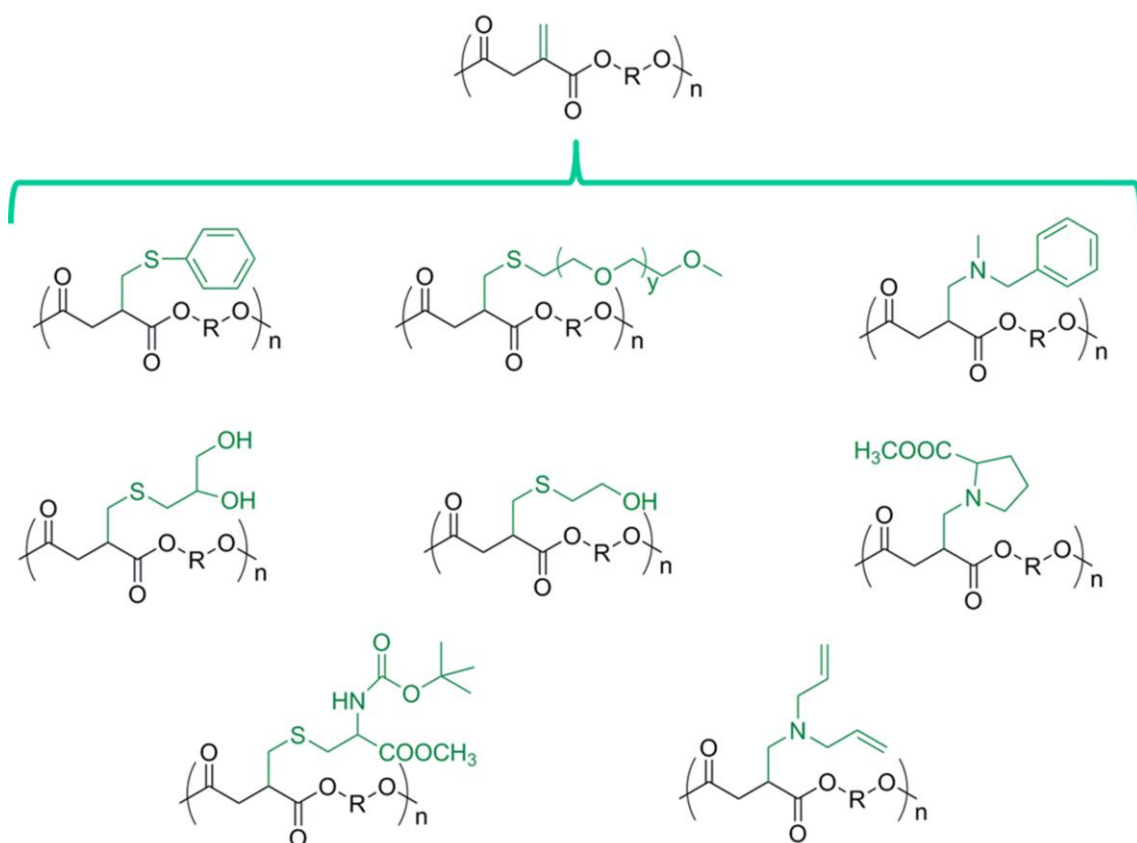
Fig. 22. Structures of the derivatives prepared using Michael addition reactions. [20], Copyright 2015. Reproduced with permission from the Royal Chemistry Society.
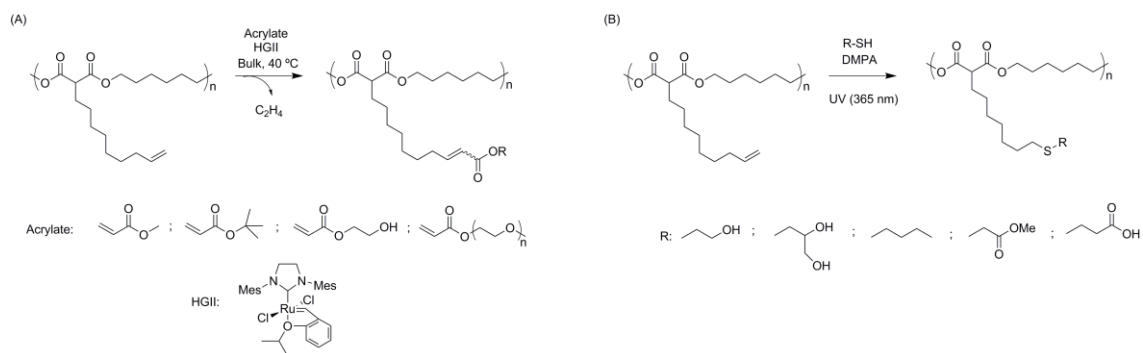
Fig. 23. Polymer-functionalization via ruthenium-catalyzed olefin-metathesis (A), and via thiol-ene addition reaction (B) [93].

UV light

UP

$H_2NCH_2CH_2NH_2$

PMMA

Crosslinking point of diamine with the maleate bonds

Crosslinking point of PMMA with itaconate bonds
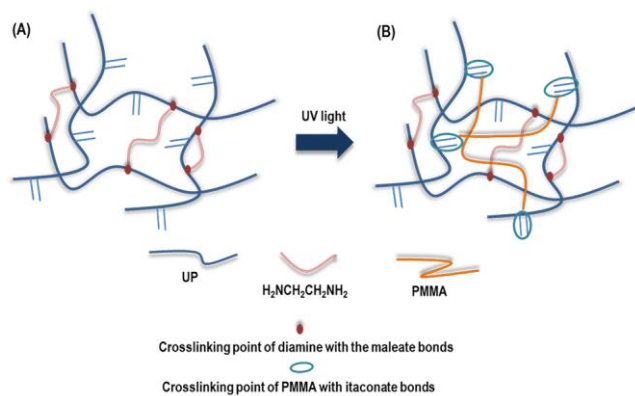
Fig. 24.   Representation of the SN (A), and of the DN (B). [95], Copyright 2015. Reproduced with permission from the Royal Chemistry Society.
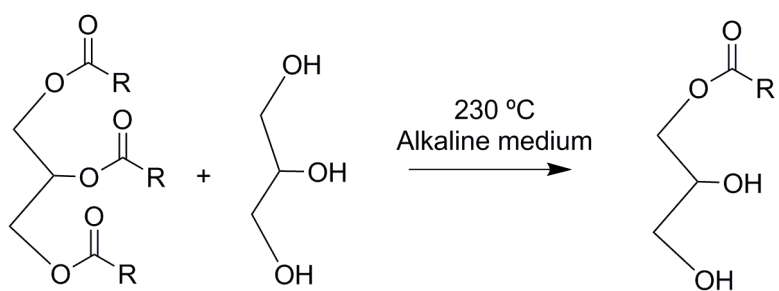
Fig. 25.   Alcoholysis reaction to yield a polyol based on vegetable oils.
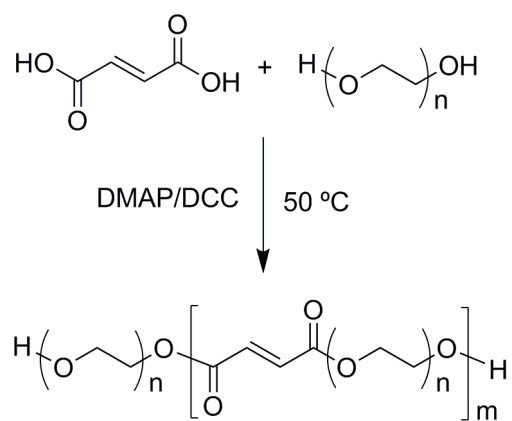
Fig. 26. Reaction of PEG with fumaric acid in the presence of DMAP/DCC [8].
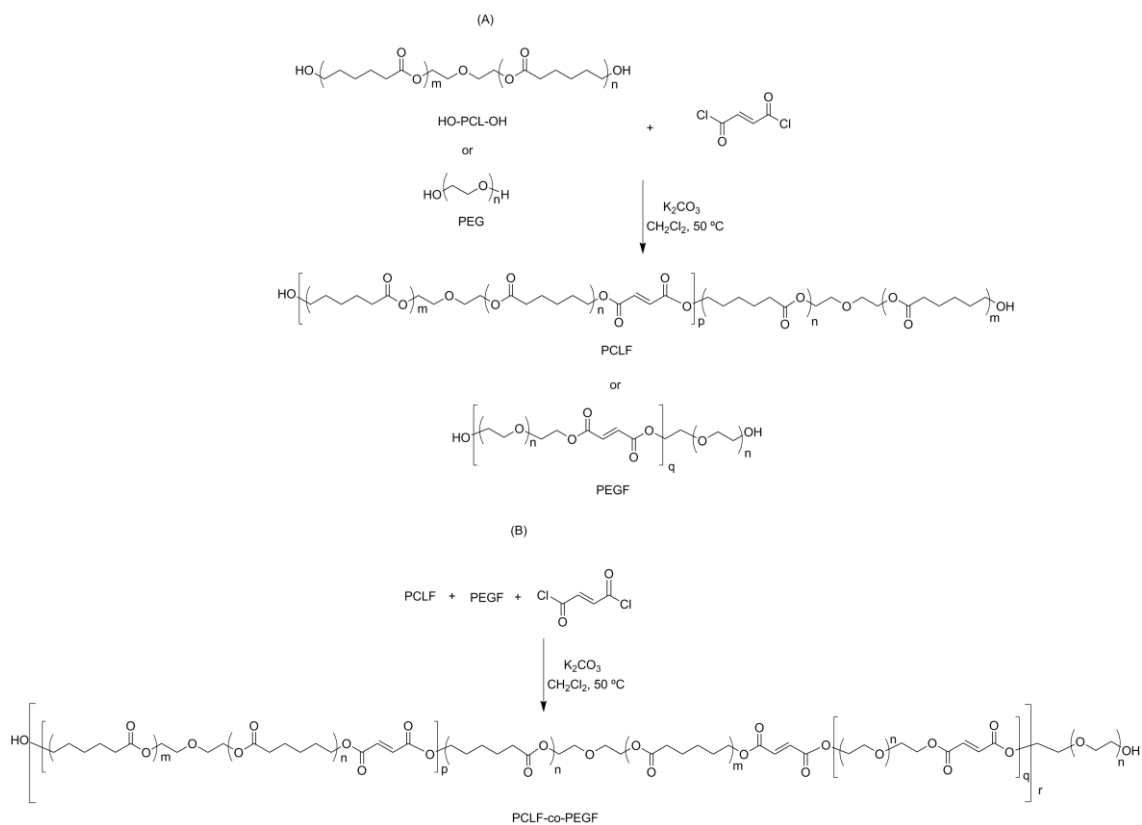
Fig. 27. Synthesis of: (A) PCLF, PEGF, and (B) PEGF-*co*-PCLF [104].
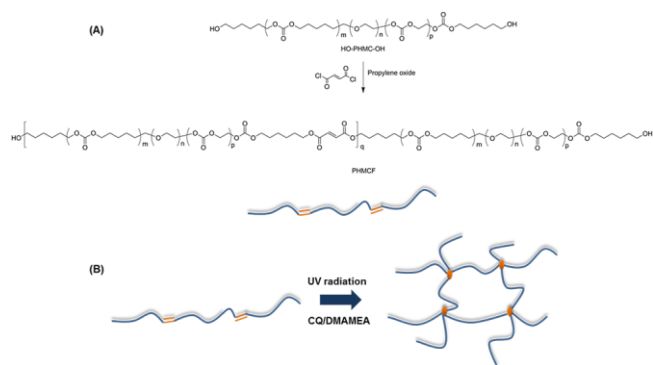
Fig. 28. Synthesis of: (A) PHMCF macromers, and (B) respective networks [107].

(A)
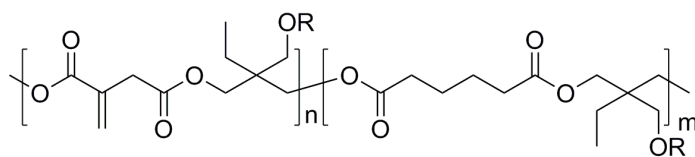
Poly(trimethylolpropane itaconate-*co*-trimethylolpropane adipate)

R: H, polymer
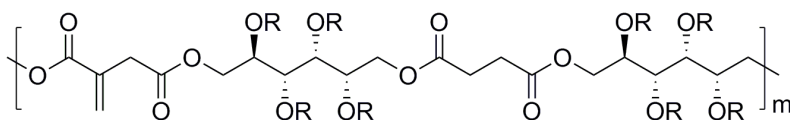
Poly(sorbitol itaconate-*co*-sorbitol succinate)

(B)
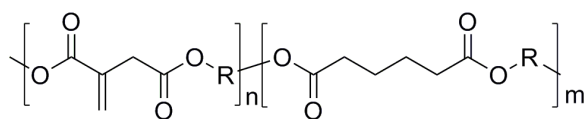
Poly(diol itaconate-*co*-diol succinate)

Fig. 29. Branched polyesters obtained by thermal polyesterification (A) and the photocurable polymer achieved by enzymatic polymerization (B) using CALB as catalyst.

(A)

Diacid I

Diacid II

Diacid III

Diacid IV

Anhydride I

Anhydride II

Anhydride III

Anhydride IV

(B)

Diacid or Anhydride   +   HO~~~~~~OH $\xrightarrow{\text{Sn(Oct)}_2}$ Polyester

160 ºC, 24 h, 30 torr

(C)

R, R': H; Me

$\xrightarrow[\text{130 ºC}]{\text{AIBN}}$ Polyester Thermoset Elastomer
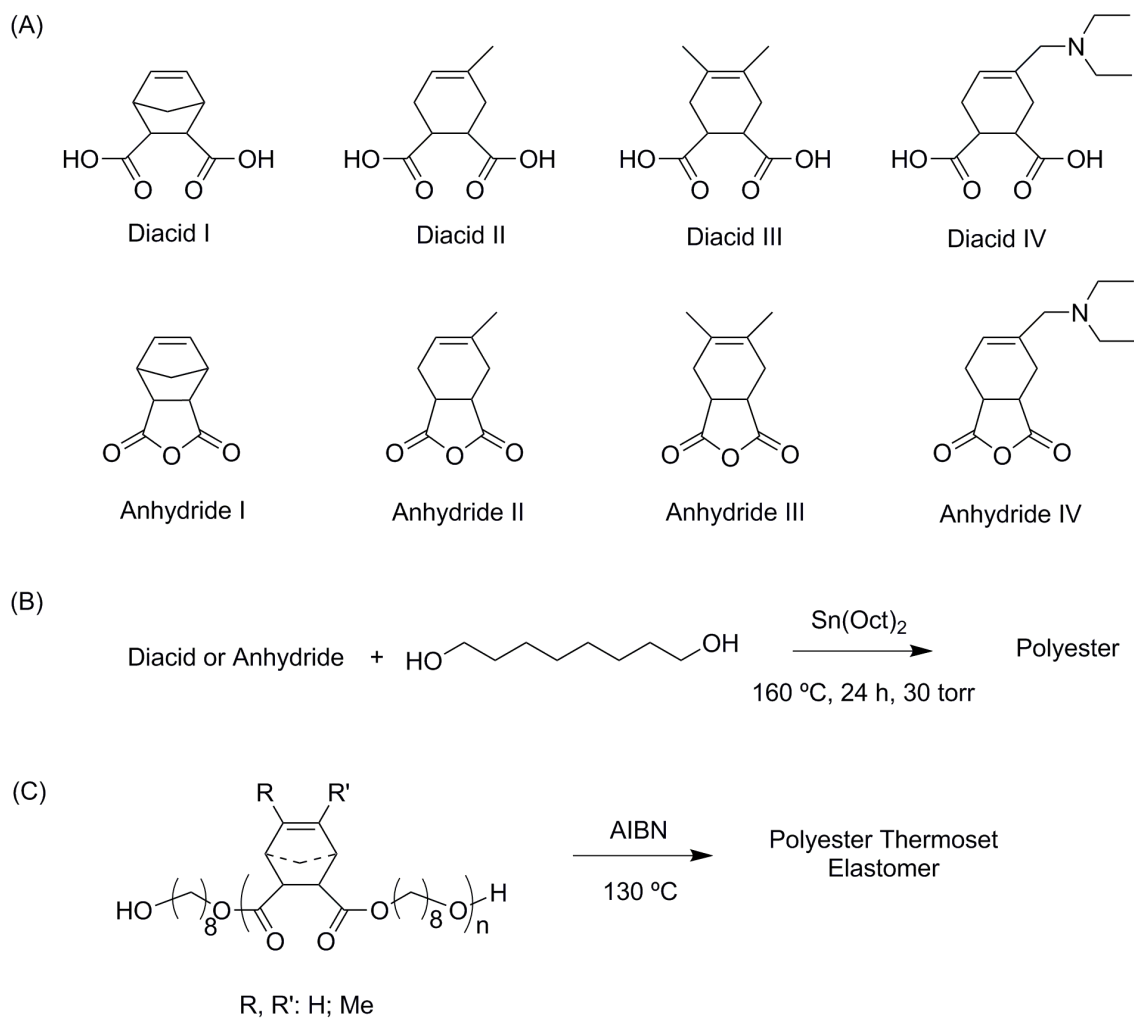
Fig. 30. (A) Unsaturated dicarboxylic monomers synthesized for the preparation of new unsaturated aliphatic polyesters, (B) overall strategy for the synthesis of polyesters, and (C) synthesis strategy for polyester thermoset elastomer [109].
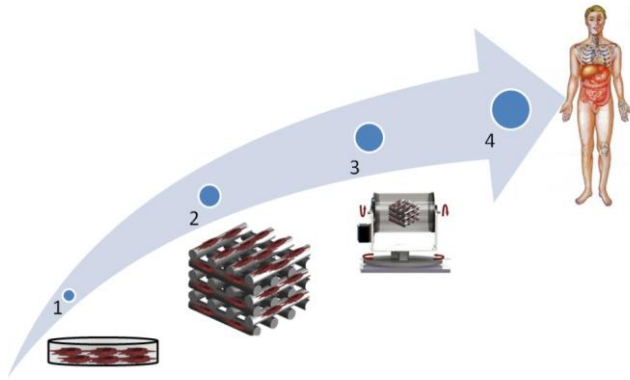
Fig. 31. Illustration of the four main steps in scaffold-based TE strategy. 1) Cell harvesting and in vitro expansion; 2) cell seeding in porous scaffolds; 3) *in vitro* dynamic cell culture using bioreactors; 4) scaffold+cell construct implantation.
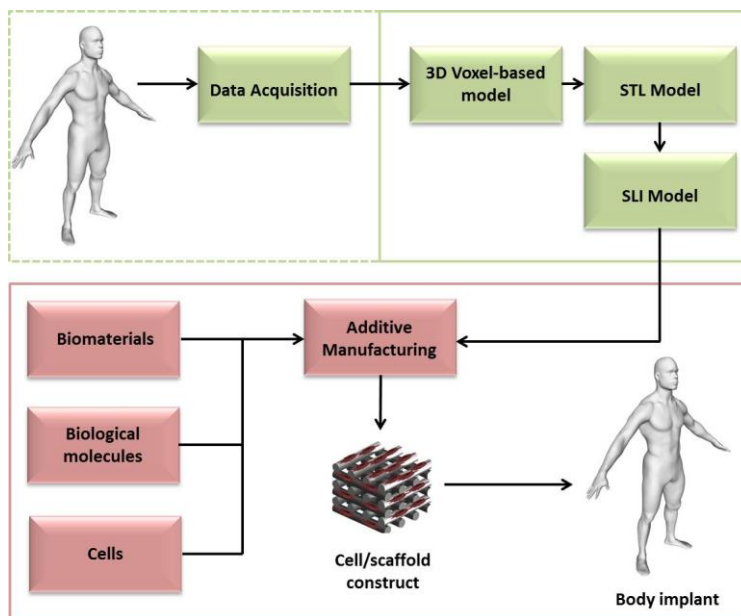
Fig. 32. Schematic representation of the main steps required to produce TE scaffolds using AM techniques. [136], Copyright 2004. Adapted with permission from WIT Press; and [141].
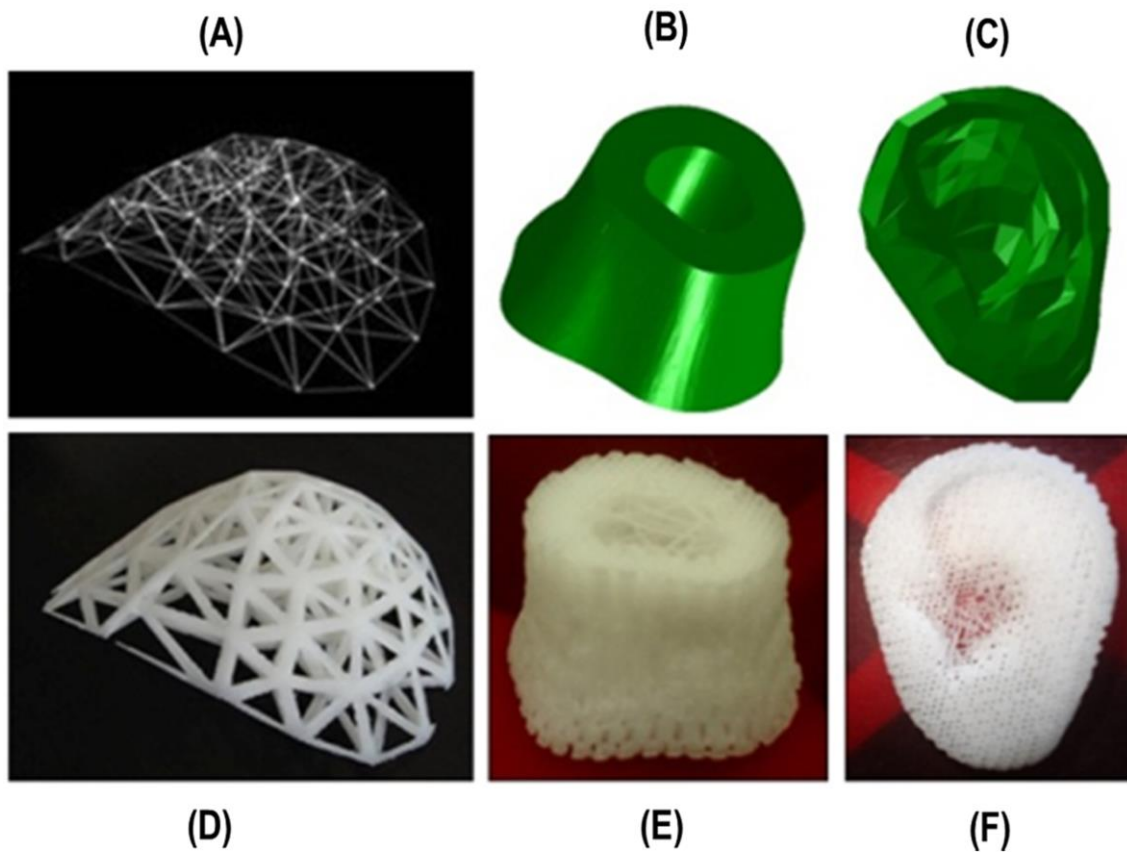
Fig. 33. 3D CAD and physical models produced via melt extrusion. (A) 3D CAD model of breast implant; (B) 3D CAD model of humerus bone; (C) 3D CAD model of human ear; (D) 3D physical model of breast implant; (E) 3D physical model of humerus bone; (F) 3D physical model of human ear. Part (d), [142]. Copyright 2012. Reproduced with permission from Elsevier Ltd.
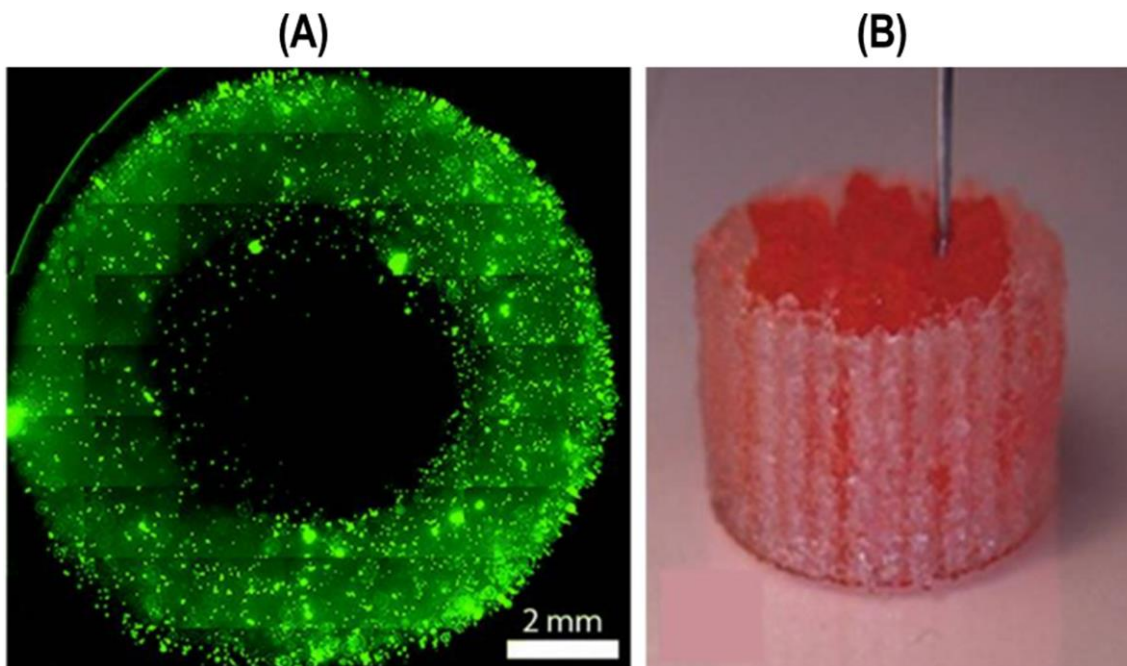
Fig. 34. Constructs with embedded cells. (A) PEG-co-PDP copolymer hydrogels with HUVECs encapsulated cells prepared via stereolithography (SLA). (B) gelatin/alginate/fibrinogen containing adipose-derived stem cells (in pink) and hepatocytes in gelatin/alginate/chitosan (white). [143] ; and Part (A), [144], Copyright 2015. Reproduced with permission from the Royal Chemistry Society.
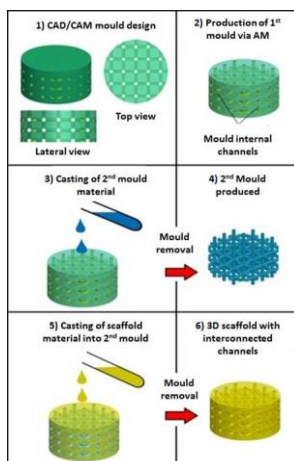
Fig. 35. Methodology for indirect fabrication of TE scaffolds. [145], Copyright 2008. Adapted with permission from Taylor & Francis.
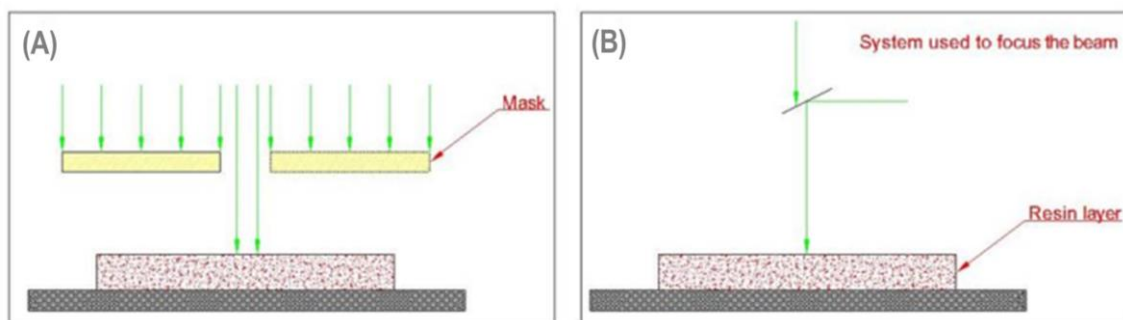
Fig. 36. Conventional stereolithography: Mask-based method (A); Direct or laser writing method (B). [148], Copyright 2011. Adapted with permission from Springer
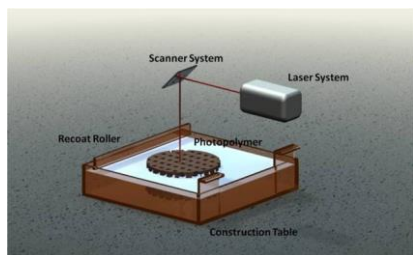
Fig. 37. Schematic representation of SLA system. [142], Copyright 2012. Reproduced with permission from Elsevier Ltd.
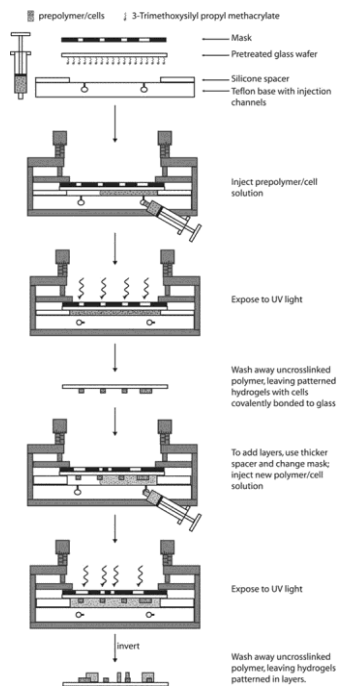
Fig. 38. Schematic illustration of the SLA process to produce micrometric resolution constructs containing cells. [154], Copyright 2002. Reproduced with permission from Springer
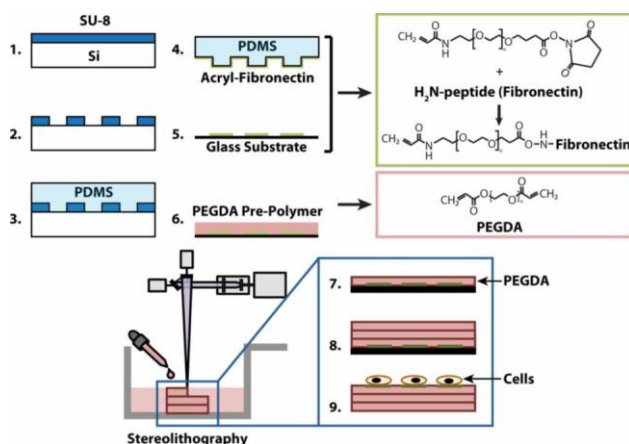
Fig. 39. Schematic illustration of the fabrication method used to create patterns of acryl-fibronectin on PEGDA hydrogels produced by SLA [. [155], Copyright 2012. Reproduced with permission from Taylor & Francis.
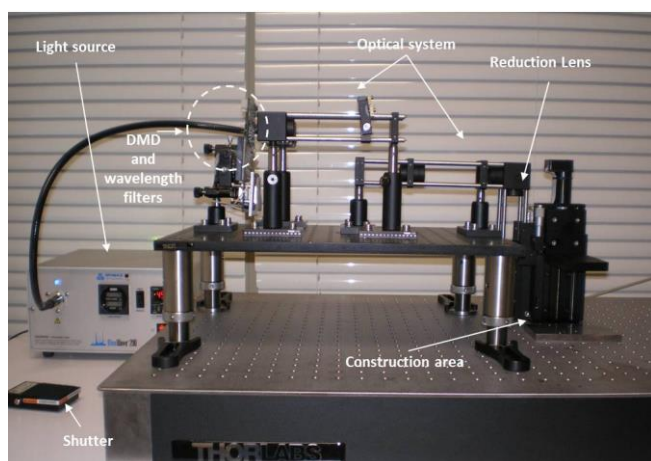
Fig. 40. Integral micro-SLA system developed by CDRSP researchers.
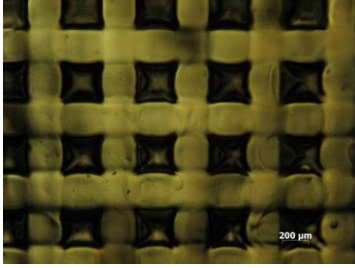
Fig. 41.   PHEMA 3D scaffold produced via SLA.
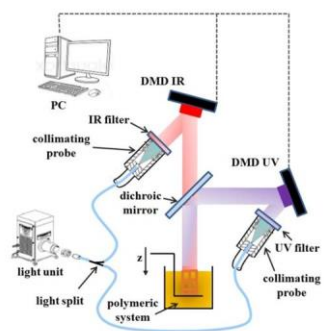
Fig. 42. Irradiation process in stereo-thermal-lithography. [148], Copyright 2011. Adapted with permission from Springe
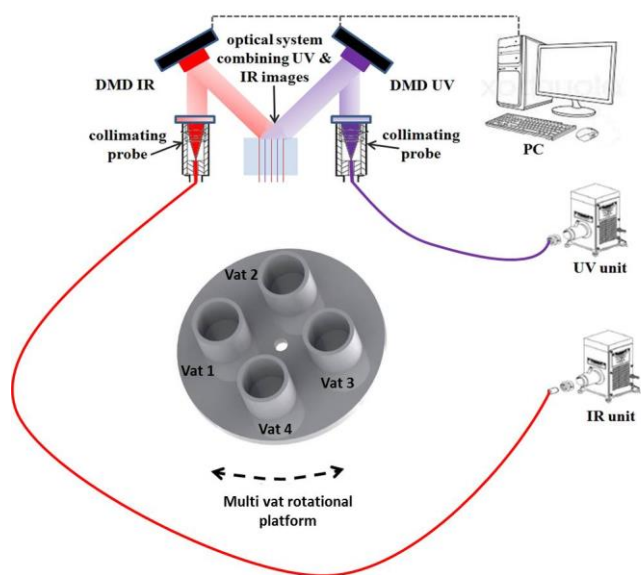
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Fig. 43.　Schematic representation of the stereo-thermal-lithography process with rotating multi-vat system which allows the production of multi-material constructs. Adapted with permission [142], Copyright 2012, from Elsevier Ltd; and [148], Copyright 2011, Springer

UP4

UP5
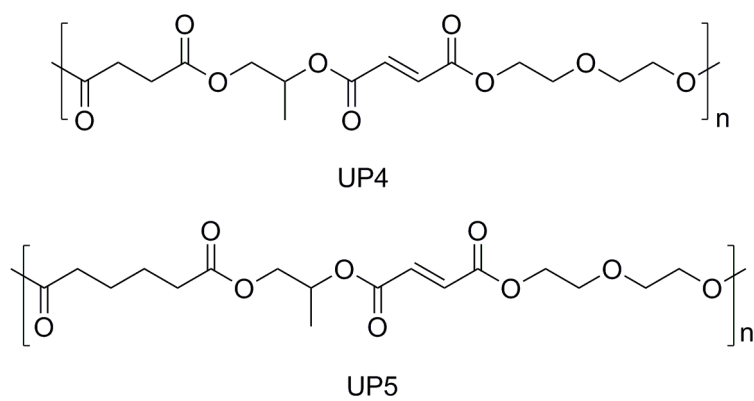
Fig. 44. Structures of the biobased UPs developed by Gonçalves and co-workers [18] that were used in the development of scaffolds by micro-STLA.
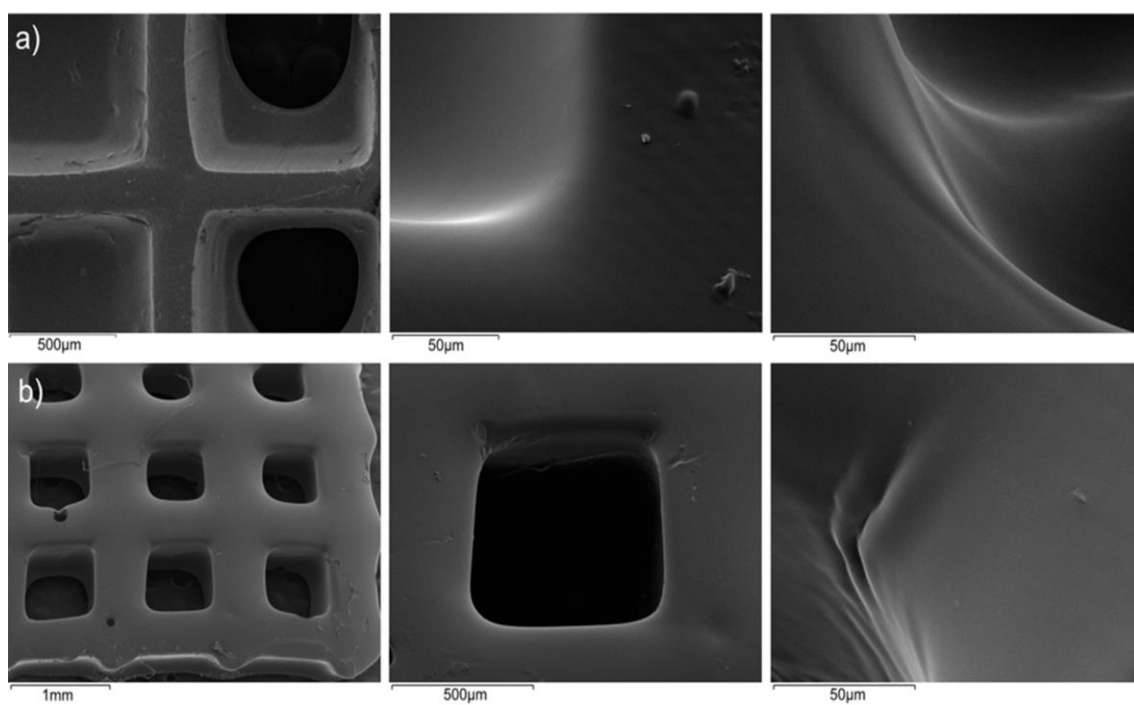
Fig. 45. SEM pictures of the scaffolds obtained by micro-SLA a) scaffolds based on UP5/Styrene, with different curing times and b) scaffold based on UP4/HEMA formulation (for UP4 and UP5 structures' see Figure 44). [18], Copyright 2014. Reproduced with permission from IOP
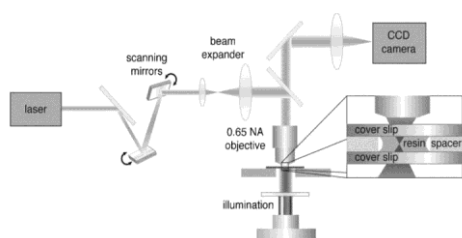
Fig. 46. A typical setup for multi-photon polymerization. [171], Copyright 2014. Reproduced with permission from John Wiley & Sons Inc.
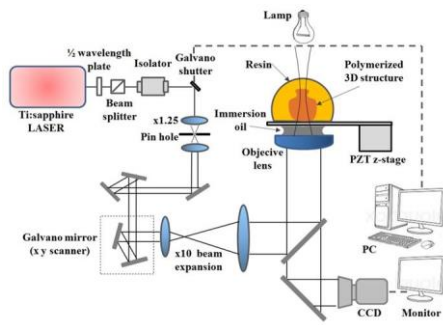
Fig. 47. Typical set-up for nano-stereolithography process. Adapted with permission from. [148], Copyright 2011, Springer; and [167], Copyright 2006, John Wiley & Sons Inc.