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Abstract

Nature inspiration is a promising tool to design new biomaterials especially for those frontier technological areas such as tissue engineering and nanomedicine. Polyurethanes (PURs) are a flexible platform of materials that can be designed to fit the requirements imposed by the final applications. The choice of their building blocks (which are used in the synthesis as macrodiols, diisocyanates, and chain extenders) can be implemented to obtain biomimetic constructs, able to mimic the native tissue in terms of mechanical, morphological and surface properties. In bone tissue engineering elastomeric PURs avoid the shear forces at the interface between bone and the implant, supporting the proliferation of osteogenic cells. Soft tissues can be engineered equally efficiently by PURs, which have been reported to be reliable candidates in the fabrication of muscle constructs (including heart), blood vessels, cartilage and peripheral nerve regeneration. This review summarizes recent progress in biomedical applications of polyurethanes. After an introduction on the concept of biomimetics (paragraph 2), the use of PURs in the engineering of hard tissues (para.3.1), soft tissues (para. 3.2) and in nanomedicine (para. 4) is reported. Taken collectively, reports in the literature clearly indicate the potential of PURs to complement or substitute alternative, FDA approved, degradable polymers such as those belonging to the polyester family in the replacement of damaged tissue or organs, as well as in the emerging field of nanomedicine where they might show superior drug encapsulation efficiency and enhanced capability to target specific tissue compartments.

Lists of the abbreviated terms

PUR: Polyurethane
PCL: Poly(ε-caprolactone)
PLA: poly(lactic acid)
PGA: poly(glycolic acid)
PLGA: poly(lactic-co-glycolide)
PEG: poly(ethylene glycol)
FDA: Food and Drug Administration
ECM: Extracellular Matrix
CUPE: cross-linked urethane-doped polyester
HA: hydroxyapatite
TDA: 2,4-toluenediamine
HMDI: 4,4′-dicyclohexylemethane diisocyanates
MDI: methylene diphenyl diisocyanate
HDI: hexamethylenediisocyanate
LTI: lysine triisocyanate
MDEA: N-methyldiethanolamine
BDEA: N-butyldiethanolamine
TIPS: thermally induced phase separation
SBF: simulated body fluid
BSA: bovine serum albumin
BMP-2: bone morphogenetic protein-2
SEM: Scanning Electron Microscopy
TCPS: Tissue culture polystyrene
ANP: Atrial nautrieutic peptide
VSMC: vascular smooth muscle cell
CPC: cardiac progenitor cell
HUVEC: Human Umbilical Vein Endothelial Cells
Ab: antibody
nps: nanoparticles
DOX: doxorubicin

PX: Paclitaxel

1. Introduction

Biomaterials are substances from synthetic and natural origin that are designed to realize devices to cure diseases, minimize suffering and support the functioning of body parts. A suture, a contact lens, a hip prosthesis, a pacemaker are all mainstream examples of medical devices which have been made realizable and marketable thanks to the progress in biomaterials' science and technology. Research in biomaterials can be dated back to ancient times since sutures or stitches used to repair wounds from linen or fibres from intestines, were used several thousand years BC. The Mayans repaired the loss or a damage of a tooth with a piece of blue nacre shell. Since then research and development efforts for metallic, plastic, and ceramic materials have made possible the replacement or repair of several parts in the body such as blood vessels (1952), hip (1961) and many others (between 1960 and 1980)[1]. In this arena, biodegradable plastics have gained also market acceptance in the medical sector, under the new concept that, in several cases, biomaterials have only to temporarily support the spontaneous repair process in the body. New areas such as controlled and targeted release of drugs and regenerative medicine were significantly boosted by new findings on degradable polymers. However, materials which were approved (e.g. polymers belonging to the polylactic or polyglycolic acids family) were originally designed for other applications and then proposed for medical use. This fate is common to all polymeric biomaterials, including non-biodegradable ones. This approach usually results in various drawbacks in the final application, e.g. the release of acidic compounds during the degradation of polylactic acid results in a lowering of the local pH at the implant site, with consequent danger of inflammation reaction and adverse body response.

Polyurethanes (PURs) are a class of materials which account for a little amount on the total of polymers used in biomedical applications. Nevertheless they show an elastomeric behavior that is well suitable for the realization of component of implanted devices, and satisfactory blood compatibility. Moreover, due to the various compositions available, belonging to both the biostable and degradable families of biomaterials, they have found diverse applications in the medical and pharmaceutical field.[2] The bad reputation of polyurethanes as biomaterials, as it is perceived by the general public and non specialized technicians, is mostly related to the potential leakage of a carcinogenic degradation product (2,4-toluenediamine (TDA) when they were used as foam coatings of the shell of breast implants.[3] Nonetheless U.S. Food and Drug Administration concluded that the use polyurethane-coated breast implants posed only infinitesimal health-risk to women with breast implants and the products remained on the market.[4] Moreover, the risk is associated only with a specific PUR composition, which, according to the application, can be designed to avoid any risk from degradation by-products in both cases of involuntary or intended degradation of the material. On the other hand, PURs have been reported to promote cells adhesion and proliferation and tissue regeneration, both in vitro and in vivo5. Due to their tunable physicochemical properties, biodegradable PURs are attractive substrates in regenerative medicine (figure 1). For instance PUR composition can be opportunely selected in function of the tissue that has to be replaced. Due to the differences in tissue mechanical properties (figure 2), rigid polyurethane scaffolds are suitable for hard tissue replacement; on the contrary soft polyurethane construct better fit the requirement of soft tissue.

2. Biomimicry in tissue engineering

Tissue engineering is aimed at the realization of *in vitro* engineered tissues/organs or the *in vivo* restoration of tissues/organs by means of a suitable three-dimensional matrix, called scaffold, able to properly interact with cell component. In any organ and tissue, the extracellular matrix (ECM) regulates the means by which adjacent cells communicate with each other and the external environment, therefore the ECM organization and composition impact organ development and function.[6] Thus, a promising approach of tissue engineering strategies is the design of scaffolds with biomimetic properties inspired by the natural ECM, able to suitably drive cell response. Scaffolds should mimic the natural ECM of the tissue/organ to be regenerated, with regard to its composition (biochemical biomimickry), structural-morphological (morphological biomimickry) and mechanical

(mechanical biomimickry) properties. ECM is generally composed of structural proteins (such as collagen and elastin), cell-adhesive proteins (such as collagen, fibronectin, vitronectin, laminin and tenascin), glycosaminoglycans (such as hyaluronic acid, dermatan sulfate, keratan sulfate, chondroitin sulfate and heparan sulfate), proteoglycans and interstitial fluid. Moreover, in mineralized tissues, such as bone, a high percentage of inorganic components (mainly hydroxyapatite) is present, and their organization is regulated by organic molecule assembly. The composition and relative amount of ECM components varies depending on the tissue, providing different biochemical and mechanical features. For instance, the relative collagen/elastin amount modulates ECM mechanical properties, providing traction resistance in the case of abundance of collagen (e.g. in tendons) or elastic properties in the case of abundance of elastin (e.g. in skin and blood vessels). On the other hand, cell-adhesive proteins influence cell behavior through the activation of integrin receptors. Integrins are transmembrane cell surface receptors which ensure communication and mediate bidirectional signalling across the cell membrane. When integrins bind to the ECM, a cascade of events named "outside-in" signaling occurs, affecting cell morphology, proliferation, and survival.[7] Finally, proteoglycans adsorb interstitial water, forming a hydrated matrix highly resistant to compression forces; moreover, they have a role in the chemical signaling between cells, as they bind cell secreted molecules, such as growth factors, proteolytic enzymes and their inhibitors, regulating their activity.

In biomimetic scaffolds, mechanical properties of the ECM are generally reproduced by the selection of proper biocompatible structural materials with mechanical properties matching those of native ECM. Figure 2 shows the mechanical properties of soft tissues as a reference for the selection of proper scaffolding materials.[8]

On the other hand, for hard tissues such as human cortical bone, the reference elastic modulus is 12-18 GPa.[9] As scaffold structural materials are degradable, their mechanical properties decrease as a function of time during *in vivo* implantation or *in vitro* cell culturing. However, they should keep mechanical integrity until cells have deposited their own ECM and do not require further mechanical support by the scaffold. The natural cell communicating activity of cell adhesive proteins in the ECM is mainly developed through short peptide sequences. For this reason, the ability of the ECM to communicate with cells can be reproduced by the use of biomimetic peptides, with which the material can be functionalised. Table 1 collects the main biomimetic peptides used for the aforementioned purpose.[10] Peptides can be grafted on the scaffold surface or included into the bulk material through the synthesis of linear polymers (such as PURs) or crosslinked hydrogels containing peptide units.[10] Alternatively, adhesive proteins can be used as scaffold coating or structural material component in biomimetic bioartificial blends.[10] Proteins provide complex signals to cells with spatiotemporal dynamics, however due to their multifunctionality they do not drive a specific cell response. In *in vivo* tissue engineering strategies with acellularised scaffolds, the use of specific peptides is thus preferred to induce the adhesion and drive the behaviour of specific endogenous cells. In the case of cellularised scaffolds in *in vivo* or *in vitro* tissue engineering strategies, peptides may be preferred to induce cell differentiation towards a specific phenotype.

Finally, scaffold architecture should be properly designed to mimic the structural properties of the native ECM. For instance, in the case of anisotropic tissues, such as skeletal and cardiac muscle, scaffolds should provide anisotropic structural (and mechanical) properties to properly guide cell behaviour. Scaffold porosity should allow cell migration within the scaffold structure, as well as inflow of nutrients and outflow of catabolites. For this reason pores should be interconnected. Moreover, pore size should be higher than the typical size of cells constituting the tissue; smaller pores with 5-10 μm size should also be present to allow endothelial cell migration (angiogenesis). Finally, as ECM structural proteins display a typical nanofibrous organization, nanofibrous scaffolds could provide morphological biomimetic properties, favouring cell attachment by their high surface-to-volume ratio.

Table 1. Biomimetic peptides, their natural source and applications in tissue engineering. Reprinted with permission.[10] Copywright 2014, Elsevier Inc**.**

Biomimetic peptide	ECM molecule source	Applications
RGD	Fibronectin, laminin, vitronection, collagen and thrombospondin	• Bone and cartilage regeneration • Neurite outgrowth • Myoblast and endothelial cell adhesion, proliferation and differentiation
DGEA	Collagen	• Platelet adhesion • Neural cell adhesion
LDV REDV	Fibronectin	• Promote the adhesion of endothelial cells and fibroblasts but inhibit platelet adhesion
IKVAV YIGSR RNIAEIIKDI	Laminin	• Neurite outgrowth
FHRRIKA	Heparin binding motif	• Cell adhesion mediated by proteoglycan receptors • Improve osteoblastic mineralization
Poly(A)	Elastin	• Proteolytic degradation mediated by elastase
APGL	Collagen	• Proteolytic degradation mediated by collagenase
VRN	Fibrin	• Proteolytic degradation mediated by plasmin

The following sections will describe the state-of-the-art for what concerns the realisation of biomimetic structures based on the proper selection of the starting material and its subsequent processing/functionalisation. The role of PUR to act as the platform of election to support the regeneration of tissues with different structural and functional properties is highlighted. The last section illustrates the potential of the use of tailor-made PUR in the emerging field of nanomedicine for cancer therapy.

3. Tissue engineering

3.1 Hard tissue

3.1.1 BONE TISSUE ENGINEERINGBone tissue engineering represents an emerging research field aimed at the development of alternative clinical treatments to allografts, autografts, and xenografts[11] for bone replacement in case of orthopaedic defects, bone neoplasia and tumours, pseudoarthrosis care, stabilization of spinal segments, and a variety of applications in maxillofacial, craniofacial, orthopaedic, reconstructive, trauma, and neck and head surgery.[12,13,14,15] Bone tissue is found in two different forms: compact bone and cancellous or spongey bone. Compact bone is a very dense tissue forms a shell around cancellous bones and is the primary component of the long bones. Mature compact bone is lamellar, or layered, in structure. Cancellous bone is found forming the core of most flat and irregular bones. It is also very prevalent in the epiphyses(ends) of long bones. The construction of spongey bone is quite different than that of compact one. Spongey bone is more open and in cross-section offers a compartmentalized appearance not unlike that of a sponge.

General properties required for scaffolds aimed at bone tissue engineering are biocompatibility, bioactivity, osteoconduction, osteoinduction, and biodegradation.[10,16] Among biomaterials, metals possess suitable load-bearing mechanical properties, whereas ceramics display biomimetic properties, but as a drawback, they are poorly degradable.[17] Polymeric biomaterials are suitable for bone tissue engineering due to their biocompatibility and degradability.[18] Natural polymers, such as proteins (or polypeptides and peptides) and polysaccharides are generally employed as

functionalising materials due to their scarce mechanical properties and high biocompatibility and biomimickry. On the contrary, biocompatible synthetic polymers are advantageous due to their reproducible chemico-physical properties and better mechanical properties. The most commonly used biodegradable synthetic polymers for three-dimensional (3D) scaffolds in tissue engineering are saturated poly(α-hydroxy esters), including poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), as well as poly(lactic-co-glycolide) (PLGA) copolymers, which are FDA-approved materials.[19,20] These polymers are degradable by hydrolytic mechanism and degradation products are not cytotoxic. However, a drawback of synthetic polymer is their poor osteoconductivity, due to the non-mimetic chemical structure, and inferior mechanical properties with respect to the ones of bone tissue. For this reason, synthetic biocompatible polymers have been generally used in polymer-ceramic composites for bone tissue engineering.[21,22,23,24] However, composites have been frequently found to display brittle behaviour due to poor interaction with the ceramic phase.[25],[26].

The synthesis of alternative synthetic polymers to traditional biocompatible poly(α-hydroxy esters) has been driven by the need of materials with controlled degradation rate, improved interaction with the ceramic phase, functionalised with bioactive molecules enhancing osteoconduction and osteoinduction properties.

3.1.1.1 Polyurethane scaffolds for bone engineering

Elastomeric PURs are promising as bone graft substitutes as they avoid the shear forces at the interface between bone and material, thus facilitating proliferation of osteogenic cells and bone regeneration.[27,28,29,30,31] For bone tissue engineering, PUR-based materials should maintain structural integrity for about 4–6 months and the time of complete degradation should not exceed 1 year to avoid prolonged foreign body reaction.[32] For instance, polyester/polyether ratio in macrodiols may be modulated to vary degradation rate. Moreover, the degradation products should not induce toxic effects and to this aim aliphatic diisocyanates are preferred over their aromatic counterparts.[33] Poly(ε-caprolactone) (PCL) diol is commonly used as a soft segment,[34] due to its biocompatibility, enzymatic degradation and slow hydrolytic properties.[35] Ryszkowska et al.[36] synthesised PUs from 4,4′-dicyclohexylemethane diisocyanates (HMDI), polycaprolactone diol (PCL diol) with molecular weight 2000 Da, ethylene glycol (EG) and using dibutyltin dilaurate as a catalyst. Three types of PUs were obtained with different ratio between the reagents: HMDI/PCL/EG 2:1:1, 2:3:1 and 5:1:4. As a result, PURs containing 17, 22 and 44 wt.% of hard segments were obtained. Previous studies have indicated that PUR containing about 50 wt.% of hard segments was optimal for the culture of human osteogenic cells.[37]

Inorganic-organic composites were studied aiming to mimic the bone composition, combining the toughness of a polymer phase with the compressive strength of an inorganic one.

Composite materials were prepared using 5-20 wt.% Bioglass®. Porous scaffolds were obtained by polymer coagulation combined with salt-leaching method using NaCl as porogen, showing higher porosity than 70 wt.% and pores with 100–400 μm in size and pore walls containing numerous micropores (with lower size than 10 μm). The high bioactivity of composite scaffolds was confirmed by the rapid formation of hydroxyapatite on the foam surfaces upon immersion in simulated body fluid (SBF). Further examples of not crosslinked PUs used for bone tissue engineering are enclosed in Table 2.[9]

PURs have also been proposed as fixation adhesives, as an alternative to currently used materials: epoxy resins, exhibit poor bonding in wet conditions, and evoke tissue necrosis due to polymerisation heat and toxic effects, while cyanoacrylates are questionable due to toxicity of some monomers, high infection rates and low shear strength.[38] Schreader et al. have recently studied a porous PUR adhesive reinforced with hydroxyapatite (HA).[39] A moisture-curing PUR was synthesized according to the reaction scheme in Figure 3. Curing of these PUR is based on the reaction between isocyanate groups with hydroxyl groups of polyols (Scheme 1, Figure 3) and water molecules due to moisture in the air and in the substrate and leading to carbon dioxide formation

(Scheme 2, Figure 4). Kaneka and Nippon Polyurethane industry located in Yokohoma, Japan developed one moisture-curing PUR composed of methylene diphenyl diisocyanate (MDI, isomers and homologues), polymeric MDI and a biodegradable polycaprolactone-based polyol (44% by weight), however, the exact chemical composition was a trade secret.[39] An ultrasound bath was employed to prepare a PUR paste through addition of water amounts. Once prepared, the paste was added with HA nanoparticles (1vol.%) and ready for brush-on application. Shear testing demonstrated that the adhesive reached its full strength 90 min after application. The PUR adhesives containing HA showed a not interconnected porous morphology (Figure 4) with an average size of 250 μm and the presence of small pores of around 5μm size. The application of a liquid dentin bonding primer to bone before the adhesive deposition significantly improved bonding strength to bone as compared to conventional cements.

To improve scaffold performance, the osteoinductive growth factor bone morphogenetic protein-2 (BMP-2) has been incorporated into scaffold for controlled release to stimulate osteoblast differentiation and promote bone formation. BMP-2 mechanism involves bone formation via different mechanisms such as osteoblast differentiation, chemoattraction, angiogenesis, and cell signaling at the initiation of fracture healing. The release of recombinant human BMP-2 (rhBMP-2) from a curable two component reactive PUR has been evaluated by Li et al.[40]

In one case, rhBMP-2 has been directly incorporated into the material before its polymerization; in an alternative approach, it has been loaded into PLGA micro-particles embedded into the PUR. The rhBMP-2 directly incorporated into PUR evidenced a significant burst release (35%) within the first 5 days followed by sustained release for 21 days.

Table 2. Main not-crosslinked PURs for bone tissue engineering. Adapted with permission.9 Copyright

Animal models	**Polyurethane scaffolds**	**Major conclusions**	**Ref.**
Iliac crest (sheep)	Porous scaffolds synthesized from HMDI, PEO-PPO-PEO, and PCL at various ratios. Pore size, 300 to 2,000 μm; porosity, 85%	At 18 and 25 months, all the defects in the ilium implanted with PUR bone substitutes had healed with new bone.	41,42
		The extent of bone healing depended on the chemical composition of the polymer from which the implant was made.	
		The implants from polymers with the incorporated calcium-complexing additive were the most effective promoters of bone healing, followed by those with vitamin D and polysaccharide-containing polymer.	
		There was no bone healing in the control defects.	
Bone marrow stromal cells	BDI with PCL films	Bone marrow stromal cells were cultured on rigid polymer films under osteogenic conditions for up to 21 days. This study demonstrated the suitability of this family of PEUUs for bone tissue engineering applications.	43
Femoral condyle	LTI with PCL-co-PGA-co-PDLLA	Extensive cellular infiltration deep to the implant and new bone formation at 6 weeks	44
Chondrocytes	Porous scaffolds synthesized from HMDI with PCL and isoprenoid	Although the covalent incorporation of the isoprenoid molecule into the PUR chain modified the surface chemistry of the polymer, it did not affect the viability of attached chondrocytes.	45
		The change of surface characteristics and the	

		more open pore structure of the scaffolds produced from the isoprenoid-modified PUR are beneficial for the seeding efficiency and the homogeneity of the tissue-engineered constructs.	

The encapsulation of rhBMP-2 with PLGA microparticles with different sizes (1.3 μm, PLGA-S and 114 μm, PLGA-L) reduced burst release and improved sustained release. Although polymerization conditions and heat development are potentially critical factors in a possible reduction of growth factor bioactivity, results showed that rhBMP-2 preserved its bioactivity. The direct incorporation of rhBMP-2 within the PUR was the most effective strategy to promote bone regeneration, as suggested by in vivo tests in rat femoral plug defects, analysed by micro-computed tomography (μCT) analysis after 2 and 4 weeks post-implantation (Figure 7). These results evidenced that early and sustained release of rhBMP-2, are critical for promoting wound healing. The versatility of PUR chemistry could allow a tailoring of PUR chemistry to promote bone regeneration, through their bulk functionalisation with specific bioactive proteins/peptides enhancing bone cell adhesion, proliferation and differentiation, such as collagen I and fibronectin, RGD and GFOGER biomimetic peptides.[46] For instance, the use of N-Boc serinol chain extender could be exploited for the coupling with bioactive peptides/proteins, after PUR synthesis.[47] On the other hand, surface functionalisation of PURs containing polyester segments could be performed using similar surface modification techniques to the ones employed for biocompatible polyesters, such as plasma treatment and surface hydrolysis.[11][48]

3.1.1.2 Injectable PU hydrogels for minimal invasive approaches

For some applications, such as the filling of contained defects where the structural bone is intact and defects in trabecular bone at non-weight bearing sites, injectable hydrogels are preferred to allow easy administration and minimally invasive procedures.[49] Although different physically and chemically in situ formed synthetic hydrogels exist, they generally lack suitable mechanical properties, controlled degradation and intrinsic bioactivity.[50]

PURs are a versatile class of block copolymers which offer the possibility to obtain both linear, branched or crosslinked biocompatible polymers with suitable degradation rate, tailored physicochemical properties, potentially functionalised with bioactive molecules to properly interact with cells. Injectable PURs have been developed for bone tissue engineering offering the possibility to be administered through a minimally invasive technique and the advantage to perfectly conform the defect shape. Generally, injectable PURs have been developed as in situ curable materials, by the reaction of a viscous isocyanate-terminated prepolymer with viscous hydrogen compound (such as water, polyols or polyamines). Critical aspects of curable injectable hydrogels are the biocompatibility of both the final material and the reactive components.[51] Moreover, the exothermic nature of the polymerization reaction may induce adverse effects in the surrounding tissue.[52,53] Finally, water diffusion into the reactive material may result in scaffold overexpansion, resulting in large voids.[54] Bonzani et al. have prepared an injectable PUR based on the reaction between two prepolymers, prepolymer A and prepolymer B (figure 5 and figure 6).[55] Mechanical properties have been slightly enhanced by the addition of tricalcium phosphate, on the other hand, osteoblast response has been significantly improved in the presence of tricalcium phosphate due to its osteoconduction and acid-buffering properties. Two components, lysine-derived reactive PURs have also been developed as injectable materials.[56] The liquid components included a terpolyester triol, a lysine triisocyanate (LTI)-poly(ethylene glycol) (PEG) prepolymer, a catalyst solution comprising triethylene diamine dissolved in , and water (both present in the reactive mixture and diffusing from the environment). Bovine mineralized bone particles were added into the PUR with the aim to improve mechanical properties, control material expansion due to the absorption of excess moisture

from surrounding tissue, and improve osteoconductivity. A kinetic model was developed to study the reactivity of the components, based on in situ attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy to calculate the disappearance of isocyanate moieties. This study evidenced that only non cytotoxic polyester triol and dipropylene glycol were released as partially unreacted. The composite was found to support cell adhesion, infiltration and remodeling in an in vivo rabbit model of femoral condyle defects. In addition, inflammatory reaction was not observed, evidencing the biocompatibility of the material and released unreacted reagents.

3.2 Soft Tissue

The term "soft tissue" refers to tissues that connect, support, or surround other structures and organs of the body, not being bone. The soft tissue definition includes tendons, ligaments, fascia, skin, fibrous tissues, fat, muscles, nerves and blood vessels.

In these tissues fibers are responsible for the tissue resistance resulting in an anisotropic viscoelastic behavior. In contrast to hard tissues, soft tissues may undergo large deformations.

The mechanical behavior of soft tissues, can be mimicked by a handful of materials from synthetic origin, especially elastomers.[57] Among these, PURs have demonstrated to be reliable candidates in their replacement.[56] Several studies reported the use of PURs in the fabrication of muscle constructs, in vascular tissue engineering, nerve repair, as reported in the following paragraphs and summarised in figure 1.

3.2.1 SKELETAL MUSCLE TISSUE ENGINEERINGSkeletal muscle may be damaged by exposure to myotoxic agents, extremely hot or cold temperature, and affected by ischemia, myopathies, the latter being characterized by a progressive waste of tissue.

When muscle structure is completely compromised or the muscle has been ablated by surgical procedure or injuries, the engineering of skeletal muscle constructs may provide a valid alternative to muscle tissue transplant.

Skeletal muscles are composed of bundles of highly oriented and dense fibers, which are closely packed together in an extracellular three-dimensional matrix, to form an organized tissue with high cell density and orientation, aimed at generating longitudinal contraction[58]. Therefore, in the design of scaffolds for skeletal muscle repair, constructs with parallel aligned fibers have to be considered in order to provide an efficient reconstruction of damaged muscle tissues.

Microfibrous polyester scaffolds have been extensively studied but have some disadvantages, mainly with respect to their high modulus and low-yield elongation.

3.2.1.1 Polyurethane scaffolds for skeletal muscle engineering

In order to designed scaffolds that mimic the mechanical properties of skeletal muscle, Riboldi and colleagues proposed the use of Degrapol™, a degradable block polyesterurethane, consisting of block of poly((R)-3-hydroxybutyric acid)-diol and blocks of poly(ε-caprolactone-co-glycolide)-diol linked with a diisocyanate.[59] Degrapol™ scaffolds were manufactured by electrospinning to achieve microfibrous structure that mimic the native tissue. The SEM micrographs of these electrospun scaffolds showed a fibrous mesh consists of fibers of about 10 μm in diameter, with a fiber to fiber distance of about 10 μm and a slight preferential orientation. These scaffolds showed satisfactory mechanical properties: a linear elastic behavior in the 0-10% deformation range and deformation at break higher than 200% and a tensile modulus around 10MPa. These values indicate that the obtained structures better match the range of elasticity of the skeletal muscle, compared to polyester scaffolds.

Furthermore, Degrapol™ membranes were coated with collagen, fibronectin and Matrigel, in order to mimic the native ECM. Coated and uncoated scaffolds were assessed in vitro by measuring C2C12 murine cell and L6 rat myogenic cells adhesion and proliferation in static culture. Cells adhered and proliferated on all scaffolds, but cell adhesion depended on cell type, and membrane coating. For instance, optical images at day 3 of culture, showed that C2C12 murine cells adhered on Matrigel coated Degrapol™ slides in a comparable way as on Tissue culture polystyrene (TCPS) especially when Matrigel was added at high concentration (130 mg/ml). Good results were also obtained with a lower concentration of Matrigel (13 mg/ml) and with collagen-coated Degrapol™ slides. After the same culture time, L6 rat myogenic cells

showed similar behavior on DegraPol slides, except for a better adhesion to collagen-coated Degrapol™ membranes. Cell culture studies performed with primary human satellite cells showed cell adhesion degree comparable to polystyrene controls for uncoated and Matrigel-coated DegraPol slides. The same authors changed some fabrication parameters in order to obtain highly oriented microfibrous scaffolds[60] and verified the influence of the fiber orientation in cell adhesion, proliferation and myotube development/arrangement. Cells grown on highly oriented membranes showed metabolic activity significantly higher if compared to cells cultured on non-oriented ones. Despite of this, cell proliferation rate was similar for all the different scaffolds. In accordance with the structure of the underlying scaffold mesh, a high degree of orientation of myotubes was observable in the case of highly oriented membranes; on the contrary, myotubes appeared more randomly distributed in non-oriented ones.

Scaffolds with oriented surface morphology can be also obtained by surface patterning techniques. This approach was selected by Shen and colleagues, in the design of 3D poly(ester urethane) grafts that may be used for *in vivo* muscle repair after surgical interventions, such as laryngopharyngectomy.[61] In this work, microchannel patterned scaffolds were produced by using a silica wafer patterned with unidirectional microchannels of 200 μm in width and 30 μm high, separated by walls with 30 μm wide. The scaffolds were surface functionalized with gelatin or silk fibroin, by using aminolysis and glutaraldheide cross-linking, in order to increase the wettability of the scaffold surfaces and made them more suitable for cell adhesion. The resulting scaffolds showed the appropriate bulk mechanical properties as well as suitable surface chemistry for cell proliferation and growth.

3.2.2 CARDIAC TISSUE ENGINEERING

Post-infarct left ventricular remodeling of myocardium includes progressive ventricular dilatation, distortion of chamber shape, myocardial hypertrophy, and deteriorating function, which if uninterrupted leads to congestive heart failure (CHF) , poor clinical outcome and mortality. Recently, several research groups have developed Tissue Engineering and Regenerative Medicine approaches for ventricular wall reconstruction surgery. For instance an attractive methodology may be to apply an elastic biodegradable patch to the infarcted ventricle that mimic the native myocardium[62] (figure 8).

The myocardium is the muscle layer of the heart. It is composed of spontaneously contracting cardiac muscle fibers which allow the heart to contract. The myocardium is charactherised by fibers oriented in a preferred direction, similarly to skeletal muscles. Post-infarct left ventricular remodeling of myocardium includes progressive ventricular dilatation, distortion of chamber shape, myocardial hypertrophy, and deteriorating function, which if uninterrupted leads to congestive heart failure, poor clinical outcome and mortality.

Recently, several research groups have developed Tissue Engineering and Regenerative Medicine approaches for ventricular wall reconstruction surgery. For instance an attractive methodology may be to apply an elastic biodegradable patch to the infarcted ventricle that mimic the native myocardium[63] (figure 8).In order to meet the mechanical demands of force-generating contractile tissue, biodegradable elastomeric polymers (e.g. polyurethanes) may serve as appropriate scaffold materials.3.2.2.1 Polyurethane scaffolds for cardiac muscle engineering

As previously described for engineering skeletal muscle constructs, the electrospinning technique showed to be a valid approach in the fabrication of scaffold characterized by fiber oriented in a preferred direction.

Liao and colleagues[64] investigated a series of PUR electrospun scaffolds and the effect of elastic modulus and fibers morphologies, coupled with electromechanical stimulation, on myoblast differentiation. The authors formulate the hypothesis that the application of a pre-developed mature skeletal muscle cell sheet can be used for myocardial regeneration, overcoming problems related to direct myoblast injection, such as ventricular arithmia. Two different polycarbonate-urethane (having tensile strength of 35 MPa and 62 MPa and elongation at break of 585% and 325% respectively) were used and fabricated as films, random or aligned electrospun fibers of varyng size (600 nm to 10 μm) and elastic modulus (0.5, 1 and 22MPa). It was observed that aligned fibers enhanced the formation of striated myotubes, significant cytoplasmatic and nucleus elongation compared to random fibers, and upregulation of contractile proteins (α-actinin, myosin,

myosin heavy chain, and troponin I). Moreover myoblast formed myotubes with higher degree of striation when cultured on softer fibers vs. stiffer fibres. Scaffolds produced using the softer PUR show no evidence of fatigue under 10% cyclic loading of 1 Hz over a period of 7 days, demonstrating their ability to withstand continuous cyclic loading. Under optimized electromechanical stimulation, the differentiated skeletal myoblasts showed a higher degree of striation and upregulation of contractile proteins compared to those cultured on aligned fibers alone. PURs used in this work are polycarbonate-urethanes, which are identified as biodurable PURs; however it should be observed that in regenerative medicine biodegradable polymers are usually preferred, in order to avoid problems related to long term implants, such as calcification. Taken this aspect into account, it is advisable that the behaviour of polycarbonate-urethane electrospun scaffolds are tested by long term *in vitro* and *in vivo* test, before considering these biomaterials for clinical application.

Rockwood and colleagues synthesized polyester-urethanes starting from polycaprolactone diol (molecular weight of 1250) 2,6-diisocyanate methylcaproate and a L-phenylalanine-based diester chain extender, and used this polymer to fabricate electrospun scaffolds (ES-PUR).[65] Either aligned or unaligned fibres were produced, in order to investigate the relationship between structural organization, cardiomyocite moprphology and organization, and expression of the Atrial nautrieutic peptide (ANP), a critical marker for the molecular phenotype. Mechanical analysis of the different electrospun constructs showed that the aligned scaffolds were stiffer and less extensible than the isotropic mats. For instance, the isotropic fibers had an elastic modulus of 13.5 ± 1.2 MPa, ultimate tensile strain of 129 ± 26%, and ultimate tensile stress of 1.53 ±0.06 MPa. In contrast, the aligned fibers showed an elastic modulus of 22.5 ± 1.7 MPa, ultimate tensile strain of 70 ± 5%, and an ultimate tensile stress of 2.84 ± 0.08 MPa. No significant difference in cell adhesion was observed for the different scaffolds compared to tissue culture polystyrene (TCPS), suggesting that those differences in mechanical properties did not affect cardiomyocyte adhesion. Cardiomyocytes seeded onto aligned and isotropic mats adopted the underlying orientation of the materials by orienting to the long-axes of the ES-PUR microfibers, as observed by Riboldi[59] and Liao[62] with myoblast cell cultures. On TCPS and on the isotropic ES-PUR cultures, cells appeared relatively unaligned, and the contractile activity of cardiomyocytes was not directionally organized. On the contrary, cells seeded on aligned ES-PUR microfibers started to contract in one principal direction with a more linear appearance, similarly to myocytes in ventricular tissue.[66] The amount of ANP expression was significantly lower in cultures grown on ES-PUR scaffolds than in those grown on TCPS. Cells grown on aligned ES-PUR had significantly lower steady state levels of ANP and constitutively released less ANP over time indicating a more mature phenotype. Cells cultured on isotropic ES-PUR adopted an intermediate phenotype between TCPS and aligned ES-PUR. Authors conclude that the physical organization of microfibers in ES-PUR scaffolds impacts both on multi-cellular architecture and cardiac cell phenotype.

A limitation of the electrospinning method is the difficulty in obtaining pore sizes that are appropriately large.[67] To overcome these problems, scaffolds prepared by the thermally induced phase separation (TIPS) method were studies; this technique offers the ability to control scaffold pore size by changing the preparation condition.[68] Wagner and coworkers synthesized different PURs and used TIPS technique to obtain scaffolds with dissimilar pore size and porosity by changing parameters such as polyurethane solution, concentration and quenching temperature.[69] The scaffold produced starting from PUR synthetized from poly(ethylene glycol)-poly(caprolactone)-poly(ethylene glycol) triblock copolymers, 1,4-butanediisocianate and putrescine as chain extender, was found to be flexible, with a tensile strength of 0.78 MPa and elongation at rupture of 157%. These values match to a certain extent those of the native cardiac tissue.[61] Due to the interesting mechanical behavior, this scaffold was applied as a cardiac patch and tested in a rat model, in subacute infarcts, which had developed over a 2 week period after coronary ligation. It was observed that PUR construct preserved LV geometry and improved contractile function, compared to that those of non-treated animals. A relevant result was the effects that patch placement had on tissue remodeling at the structural level: smooth muscle bundless were found in the tissue below the patch and favorable alteration in myocardial wall thickness and compliance was observed. It was postulated that mechanical reinforcement provided by the polyeter urethanes material could prevent further LV dilatation, which

subsequently increases LV wall tension and improve wall thickness, and might reduce LV wall stress. Histology analysis revealed that scaffolds were most completely degraded after 8 weeks of implantation, avoiding possible complication associated with non-degradable materials (e.g. calcification). The small particles that might be generated by PUR hydrolysis are phagocytosed by the macrophage, which may be the responsible of the released of Basic Fibroblast Growth Factor and Vascular endothelial growth factor, overexpressed around the capillaries close to the patch area, as observed by immunohistochemical staining. The local release of angiogenic factors might positively influence tissue remodeling and are probably an additional reason of the functional benefit obtained with the patch.

Nonconventional techniques, such as rapid prototyping or technologies, have been introduced quite recently, since they allow a more precise control over pore size, shape and interconnectivity. In a recent work, Chiono et al.[70] fabricated a three-dimensional porous poly(ester urethane)scaffolds for myocardial repair. The fabrication method was a melt-extrusion additive manufacturing technique, that was set successfully to avoid any PUR thermal degradation. Scaffolds highly reproduced computer-aided design geometry and uniaxial tensile and cyclic mechanical tests performed on scaffold evidenced an elastomeric-like behaviour, which is promising for applications in the field of contractile tissues. Scaffolds were then seeded with cardiac primitive CD117-positive cardiac progenitor cells (CPCs), which are responsible for physiological tissue homeostasis and regeneration. CD117-positive CPCs were found to adhere to the scaffolds and to spread on their trabeculae. However, quantitative evaluation of cells behaviour in contact with scaffolds showed that they did not proliferate after 14 days of culture time, suggesting the need of a further functionalization with bioactive peptides/proteins of the natural cardiac extracellular matrix in order to obtain a more cell-friendly environment.

To sum up, for what concerns skeletal muscle and cardiac tissue engineering, satisfactory results were obtained with aligned microfibrous scaffolds produced by electrospinning and TIPS technique. However, in order to translate the fabrication of a tissue engineering construct from the laboratory to the industrial scale, additional effort is ncessary to obtain similar devices, using an alternative more reproducible and time-saving approach, such as that based on rapid prototyping.

3.2.3 VASCULAR TISSUE ENGINEERINGVascular tissue engineering is a relevant research field aimed at elaborating innovative solutions to overcome the drawbacks related to the use of conventional blood vessel substitutes, especially referring to small-diameter grafts, were synthetic materials that are successful for large-diameter grafts, failed.[71]

Blood vessels are part of the circulatory system and function to transport blood throughout the body.

All blood vessels have the same basic structure. The largest blood vessels (arteries and veins) have a thick, tough wall of connective tissue and many layers of smooth muscle cells. The wall is lined by an exceedingly thin single sheet of endothelial cells, the endothelium, separated from the surrounding outer layers by a basal lamina. In the finest branches of the vascular tree (capillaries and sinusoids) the walls consist of nothing but endothelial cells and a basal lamina, together with a few scattered pericytes. These are cells of the connective-tissue family, associated to vascular smooth muscle cells that wrap themselves round the vessels.[72]A bioengineered graft needs a careful selection in order to avoid the problems related to thrombogenicity and poor vasoactivity. Moreover scaffolds should encourage the adhesion and proliferation of endothelial cells, vascular smooth muscle cells, and fibroblasts. For instance, the proliferation of vascular smooth muscle cells (VSMCs) on artificial construct is crucial, since they are the major cells in the vascular tissue and play a key role in morphogenesis of the blood vessels. The differentiated state of the VSMC is characterized by specific contractile proteins, ion channels, and cell surface receptors that regulate the contractile process and are thus termed contractile cells. In addition to these normal functions, in response to injury or during development, VSMCs synthesise extracellular matrix proteins, become migratory and proliferate. This phenotype has been termed “synthetic cells”.[73]

The graft mechanical properties must be similar to those of a native blood vessel in order to prevent compliance mismatch at the site of anastomosis, which is one of the major reasons for graft failure.[74] To be

mechanically functional, the scaffolds should possess the strength to withstand blood vessel pressure and provide adequate suture retention strength and, in the meantime, be soft and elastic.[75]

3.2.3.1 Polyurethane scaffolds for vascular engineering

In order to designed scaffolds that mimic the mechanical properties of blood vessel, Day and colleague developed cross-linked urethane-doped polyesters (CUPEs), a new generation of biodegradable elastic polymers based on poly(1,8-octanediol citrate), and used this PUR to produce a tubular biphasic structure similar to blood vessel[76,77]. The scaffolds were found to exhibit similar tensile strength, tunable burst pressure, and suture retention properties as native veins and arteries. CUPE showed similar to or a little better thromboresistance than poly-L-lactic acid, a FDA approved material. The authors suggested that this property of CUPE may be attributed to uses of citric acid, one of the monomers used in the synthesis of the PUR, which is a known anticoagulant. Flow cytometry analysis on CUPE and poly-L-lactic acid revealed similar degrees of leukocyte activation, but inflammatory cytokine release analysis showed lower cytokine concentrations in CUPE samples, indicating a less intense inflammatory response elicited by CUPE compared to poly-L-lactic acid. These results made CUPE based scaffolds interesting. However this work lacks in cell adhesion/proliferation test, which are necessary before considering any scaffold as a good candidate for vascular tissue engineering.

In a work of Jia et colleagues[78] biomimetic scaffolds for vascular tissue engineering were produced by blending a PUR with four different types of proteins, namely collagen, gelatin, fibrinogen, and bovine serum albumin (BSA). Nanofibrous scaffolds were manufactured by electrospinning technique, in order to produce constructs having high surface area to volume ratios, for adequate cell incorporation and nutrition perfusion, and controlled fiber diameters to mimic the fibrous architecture of ECM. The electrospinning technique was selected as one of the most effective methods for preparing biocomposite scaffolds with tailored properties.[79] SEM analysis revealed fiber diameters in the range of 275-245 nm for PUR/Collagen, PUR/Gelatin, PUR/Fibronectin, and PUR/BSA composites and uniform distribution of pore sizes for all blends, except for PUR/BSA. Compared to the tensile properties of native coronary artery, the PUR/protein scaffolds had sufficient tensile strength and elastic modulus to be employed as a vascular graft. High coverage of SMCs on PUR/Collagen and PUR/Gelatine nanofibers was observed over 6 days of culture and the scaffold supported cell alignment over its surfaces; cells exhibited a spindle-like morphology, actin filament organization, and MHC expression specific of VSMCs, suggesting both electrospun PUR/Collagene and PUR/Gelatine scaffolds as promising substrates for application as functional blood vessels.

A further step in the development of a scaffold architecture mimicking that of a blood vessel, may be the achievement of aligned nanofibrous scaffold, which can induce VSMCs alignment. It is well-known that in the medial layer of the natural arteries, VSMCs and collagen fibrils have a marked circumferential orientation so as to provide the mechanical strength necessary to withstand the higher pressures existing in circulation. Moreover, not only the structural integrity but also the vasoactivity is dependent on the VSMCs and fiber orientation in native arteries.[80] In this contest, Wong and colleagues produced aligned electrospun PUR, collagen-blended and elastin-blended PUR scaffolds.[81] The PUR was a polyether-urethanes, for instance poly[4,40-methylenebis(phenyl isocyanate)-alt- 1,4-butanediol/polytetrahydrofuran]). Blends were designed on the hypothesis that incorporating collagen and elastin could confer better properties such as tensile strength and elasticity matching those of the native arteries, as well as provide biological cues to enhance biocompatibility. Scaffolds made of PUR-protein blends were crosslinked by using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide to prevent the dissolution of proteins in aqueous solutions. However, in this work no characterization was conducted in order to verify that the cross-linking reaction was accomplished. The resulting PUR scaffold was strong and with excellent flexibility, as shown by its peak stress of about 33 MPa and a strain of 247%. The Young's moduli of native arteries range between 9 and 12 MPa indicating that they are softer and less elastic than unmodified PUR. The addition of elastin softened the PUR and significantly reduced the peak stress and strain to values closer to native tissues. On the contrary, collagen increased the stiffness of PUR. This work demonstrated that electrospun PUR-elastin scaffolds had viscoelastic properties that could be useful for vascular graft reconstruction. In order to better match the

mechanical properties of native blood vessel, alternative PUR formulations might advisable. For instance, aliphatic polyesterurethanes are usually softer and have a favourable *in vivo* biocompatibility, not being affected by environmental stress cracking and calcification, which is typical for polyeterurethanes.[82] Cytotoxicity and proliferation studies demonstrate that the crosslinking process did not affect biocompatibility, since there was no difference in cell viabilities between the crosslinked scaffolds and their non-crosslinked counterparts. VSMCs cultured on PUR and PUR-proteins (crosslinked) scaffolds demonstrated an aligned distribution, and proliferation in the direction of the fibers. PUR-collagen blends showed higher cell growth of VSMCs, compared to the unmodified PUR and PUR-elastine scaffolds. There was no difference in VSMC proliferation between PUR blended with 5 % elastin and PUR scaffolds. Immunostaining of the cells indicated that most of the VSMCs cultured on the PUR and protein blended PUR scaffolds exhibited the contractile phenotype, the same of the majority of VSMCs in healthy blood vessels. To sum up, different studies demonstrate that by blending PUR with ECM proteins, vascular grafts with attractive properties can be achieved. However in order to obtain constructs which possess mechanical properties similar to native tissue and, in the meantime, show excellent biological response, different PUR-collagen blends should be studied, by selecting different PURs (as suggest above) or various weight ratios of PUR to collagen

A different functionalization approach were proposed by Dubey and Mequanint, that studied a surface modification of highly porous polycarbonate-urethanes scaffolds with fibronectin[83] since, in previous works, they observed cells detachment from the scaffold, in the absence of a protein coating.[84,85,86] Fibronectin was selected as it has been shown to induce the synthetic phenotype of VSMCs, which, as previously described, is responsible for ECM secretion, required for tissue regeneration. This work demonstrated that in short-term culture, fibronectin conjugation have significant advantages for rapid VSMC distribution throughout the scaffold.

An alternative approach, used to enhance the biocompatibility of PURs, was their modification including hydrophilic PEO and sulfonate (SO_3) groups in their composition. Han and colleagues prepared sulfonated poly(ethylene oxide) (PEO)-grafted PURs (PUR-PEO-SO_3) by both surface and bulk modifications. These PURs showed excellent blood compatibility, due to several combined factors, including hydrophilicity, dynamic mobility of pendant PEO chains, and electrical repulsion of the negatively charged sulfonate groups.[87,88,89] Because of the proven benefits of PUR-PEO-SO_3 in blood-contacting area, a vascular PUR graft(obtained from Pellethane 2363-80AE) was coated with PUR-PEO-SO_3 and subjected to in vivo tests using a canine model[90]. After 24 days from implantation, a significant difference was observed between the coated and uncoated implants, with the PUR-PEO-SO_3-coated surface being much smoother. Platelet adhesion and thrombus formation appeared to be much more significant on the uncoated surface. After 39 days from implantation, the PUR-PEO-SO_3-coated grafts showed excellent crack-resistance. These results suggest that PUR-PEO-SO_3 has more blood compatibility than PUR. The amount of calcium on the retrieved samples resulted substantialy lower on the surface-modified implants, indicating that PUR-PEO-SO_3 can be an efficient calcification-resistance material. Although no mechanical failure was observed in vascular grafts during implantation in a dog for up to 39 days, the authors acknowledged that longer follow-up studies where needed, paying special attention on the mechanical stability of the implanted devices.

In conclusion, PURs results promising materials in vascular tissue engineering, mainly for their mechanical property. Different functionalization approaches were studied, showing the positive effect on smooth muscle cell adhesion of ECM proteins blending or grafting. Moreover the use of specifically designed monomer in PUR synthesis, result an interesting tool to enhance PUR thromboresistance properties, which is a key factor in vascular application.

However long term in vivo study had to be conduct, in order to assure the constructs performance and stability.

3.2.4 NERVE TISSUE ENGINEERING

Peripheral nerve regeneration is a challenging scientific field with relevant clinical and socioeconomic implications, since nerve injuries may seriously compromise patient's quality of life leading to a lifelong

function impairment and pain. Peripheral nerve consists of bundles of nerve fibers surrounded by connective tissue called *epinerium*. Each fascicle (bundle of nerve fibres) is enclosed in the *perineurium*, a sheath of less dense tissue. The individual nerve fibres are surrounded by *endoneurium* composed by connective tissue while the single axon is enclosed in the myelin sheath formed by Schwann cells which are the glial cells of the peripheral nervous system and are fundamental to regulate axons function and survival.

Transected peripheral nerve fibers are able to regenerate reaching functional recovery when an appropriate microenvironment is provided. Severe trauma resulting in abundant substance loss require bridging surgical techniques in which the proximal and distal stumps are reconnected using graft of biological or artificial origin.

Nowadays, innovative approaches in this field are focused on the development of devices and materials able to achieve clinical results comparable with the current gold standard (autologous nerve graft) reducing the associated drawbacks such as limited sources of donor nerve, the need for a second surgery to obtain the donor nerve, loss of nerve function in transplantation, and mismatch between the injured nerve and donor nerve[91].

Biodegradable nerve guidance channels (NGCs) represent a promising alternative to current clinical nerve repair procedures and the use of internal fillers within the hollow guide is a widely studied approach to enhance the regeneration in case of long gaps (higher than 3 cm in humans).[92,93]

To be suitable as a NGC material, polymers should be engineered to possess elastomeric properties and degrade at a defined rate without interfering with the regenerating environment. Multi-block copolymers offer several advantages compared to other synthetic polymers (such as PCL, PLA, PLGA) in terms of tailoring of mechanical properties and degradation rate in biological environment and easy processing.

3.2.4.1 Polyurethane Scaffolds for peripheral nerve engineering

A set of polyester urethane multi-block polymers was synthesized and processed into NGCs showing promising in vivo results.[94,95,96,97,98] The importance of NGC material properties to the regeneration process was investigated by varying PUR blocks. PUR characterized by different mechanical behaviors and degradation rate showed to strongly affect the regeneration rate and the functional recovery.

Borkenhagen et al.[93] synthesized an innovative polymer consisting mainly of two different blocks: a non-crystallizable block of poly[glycolide-co-(ε-caprolactone)]- diol and crystallizable blocks of poly[(R)-3-hydroxybutyric acid-co-(R)-3-hydroxyvaleric acid]-diol (PHB). Mechanical properties were modulated varying the crystalline domains from the crystallizable blocks while non-crystallizable blocks allowed the incorporation of “weak links” able to tailor the degradation rate of the polymer without significantly affecting its mechanical properties.

A set of three polymers were tested, differing in the content of crystallizable blocks (41, 17, and 8 wt%). The synthesized polymers were extruded into polymeric tubes at given diameters and wall thicknesses and implanted across an 8 mm gap of an axotomized sciatic nerve in the rat. Highest mean value for the number of myelinated axons as well as nerve cable area was detected in nerves regenerated through NGCs fabricated using intermediate amount of PHB blocks (17%).

The key role of mechanical properties of nerve guides in the repair of peripheral nerve defects was investigated by the authors highlighting that stiff guides based on PCL can exert tension at the suture sites in response to body movements, causing guide detachment and negatively affecting nerve regeneration (figure 9).[99]

To overcome this limitation, authors developed a biocompatible elastomeric PUR containing PCL soft segments for the production of melt-extruded guides with reduced mechanical rigidity as compared to those based on pure PCL.[94] The developed PUR, as known for synthetic polymers, lack in biological properties and do not present specific sites for cellular adhesion. Haptotactic cues were imparted to PUR by coating PUR surface with gelatin (G) or polylisine (PL). In vitro cell tests using S5Y5 neuroblastoma cells (S5Y5-NC) and neonatal olfactory bulb ensheating cells (NOBEC) were performed reporting an enhancement of the regeneration ability for PL-coated PUR. Due to their superior in vitro performance, 1.8cm long defects in the

rat sciatic nerve were treated using PL-coated PUR guides, obtaining promising results in terms of functional recovery and fibres regeneration.

Multi-layer NGCs were fabricated to impart different properties at the inner and outer side of the conduits.[100] A novel double nozzle, low-temperature, deposition manufacturing (DLDM) system was developed for PUR-collagen conduits preparation where the outer layer was composed of an elastomeric PUR, based on polycaprolactone, polyethylene glycol and 1,6-hexamethyl diisocyanate having good mechanical strength, easy processing, and a controllable degradation rate, while the collagen inner layer supported cell attachment and proliferation.[95] Thanks to the PUR easy processing, an outer layer with micropores was fabricated by combining DLDM system with phase separation. Micropores with pore size ranging from 15 to 25 μm were obtained. The presence of micropores in the outer layer is essential to allow sufficient diffusion of nutrients and oxygen from the outside of the conduits guaranteeing cell survival inside the guide.

3.2.5 CARTILAGE TISSUE ENGINEERINGArticular cartilage consists mainly of type II collagen produced by resident chondrocytes and is characterized by four zones having different composition, structure and consequently biological roles.The superficial zone contains the highest proportion of collagen fibres arranged parallel to joint and its function is to resist to the shear stress at the joint interface; the middle zone is composed by randomly oriented collagen fibres and proteoglycans that are fundamental for cartilages highly stable hydrated structure; the deep zone is characterized by collagen fibres aligned perpendicular to the surface and the calcified zone, serving as an interface layer between the non-mineralized cartilage and the underlying subchondral bone, is involved in load-bearing forces distribution.

Adult articular cartilage exhibits limited regeneration capacity, and after injuries or lesions proper treatments are necessary to limited progressive damage and osteoarthritic joint degeneration.

Tissue engineering approach is a very effective strategy to repair cartilage and promising results were obtained implanting multifunctional construct made of i) a cellular component (such as chondrogenic cells); ii) a scaffold which provides a physical environment supporting the cellular component, and iii) biological stimuli (haptotactic and/or chemotactic cues).[101, 102]

Highly hydrated polymeric hydrogels characterized by a three-dimensional structure intended to provide support for chondrogenic cells and to preserve their native phenotype were developed, but they showed poor mechanical properties resulting in difficult handling and diffuclties in maintaining their shape and structure both in vitro and in vivo.[99, 100] Biodegradable polymeric scaffolds based on synthetic polymers can overcome the drawbacks and limitations of hydrogels in terms of mechanical properties and usability.

3.2.5.1 Polyurethane scaffolds for cartilage engineering

Among the various synthetic polymers (such as poly(lactic acid), poly(glycolic acid), poly (e-caprolactone)), biodegradable PURs have shown to be advantageous for cartilage repair scaffold preparation thanks to their versatile chemistry that allowed tailoring of chemical composition and mechanical properties. However, they are associated with the lack of specific cellular properties resulting in low cell-seeding efficiency, non-homogenous cell distribution, increasing cell dedifferentiation over time, and poor surface-adhesion of newly developed ECM[103]. To face this problem and achieve the goal of cartilage regeneration, different PUR were studied and combined with natural based hydrogels and/or growth factors.

For instance, Eyrich et al. demonstrated the improvement of in vivo cartilage repair when polycaprolactone-based PURs where associated to long-term stable fibrin hydrogels.[104] Briefly, primary bovine chondrocytes were suspended in long-term fibrin glue and injected into highly porous PUR scaffolds. The obtained cellularised scaffolds were pre-cultured in vitro for 4 weeks and then subcutaneously implanted into nude mice. After 6 months in vivo, the formation of cartilage tissue with homogenous cell distribution was observed showing a significant increase in tissue formation, when chondrocytes are injected into the polymeric scaffold using a fibrin hydrogel.

Alternatively to cellularised scaffolds and in view of clinical applications, cell-free scaffold having a controllable and precise factor release are advantageous from production, therapeutic and commercial points of view. Segmented polyurethane/polylactid-co-glycolic acid by-layered scaffold for local delivery of transforming growth factor β1(TGF-β1) or bone morphogenetic protein-2 (BMP-2) were developed by Reyes

et al.[105] The use of PUR guaranteed easy processability and efficient bi-layered scaffold fabrication process. Furthermore, constant in vivo release rate of both TGF-β1 and BMP-2 was achieved within 6 weeks and the formation of good quality hyaline cartilage was observed in New Zealand rabbits after 24 weeks.

Lastly, recent studies reported the covalent incorporation of isoprenoid molecules into the backbone of biodegradable aliphatic PUR.[106] The presence of isoprenoid has a beneficial effect on chondrocytes-PUR surface interactions and improve the formation of articular cartilage tissue in vitro.

4. Nanomedicine

The term nanomedicine is defined as the medical application of nanotechnology and covers a vast array of therapeutic and diagnostic tools properly engineered at the nanoscale.[107] Nano-size drug delivery vehicles, such as polymer nanoparticles and micelles, are the most common nanomedicine tools to improve the delivery kinetics and the specificity of cytotoxic drugs, since they offer several advantages over traditional delivery in terms of maximization of therapeutic potential and minimization of side effects. Nanoparticles are known to provide (i) drug protection from degradation, reducing administered doses; (ii) passive targeting, that is the ability to leak through the defective vascular endothelium of tumors and inflammatory sites through the so-called "Enhanced Permeability and Retention Effect" in virtue of their small size (ii) enhanced control over drug release kinetics, and (iv) vast array of surface modifications to enhance targeting ability (active targeting).[105]

4.1 Polyurethanes in nanomedicine

Due their versatile chemistry, the application of PURs to the preparation of nano-structured drug delivery and targeting devices, such as nanoparticles (nps) and micelles, opens-up interesting perspectives in nanomedicine.[108] Nevertheless, little is reported in literature concerning their use as carriers for controlled drug release. Some authors described the preparation of biocompatible PUR nps via polymerization methods, which involve the in situ polymerization of starting reagents, while very few authors reported the preparation of PUR nps from pre-formed polymers.

For instance Zanetti-Ramos and co-workers reported the preparation of PUR nps via the emulsion polymerization method, which involves in situ polymerization of monomers. [109,110] They successfully obtained nps of relatively small size, ranging from 260 to 460 nm, by using non-toxic solvent and surfactants. Nevertheless, the ability to encapsulate and release drugs from such systems has not been demonstrated.

Morral-Ruiz et al. recently reported the preparation of antibody (Ab)-functionalized PUR-urea nps via the interfacial polymerization method, by using mild reaction conditions and biocompatible starting reagents.[111,112] They used polyoxyethylene 20-sorbitan monooleate (P80) as surfactant, saturated medium chain triglyceride as the oil component and isophorone diisocyanate as monomer. Streptavidin protein was added to the monomer solution, in order to introduce surface functionalities to be coupled to biotinylated antibodies. Two different Abs, able to recognize the inflammation related vascular cell adhesion molecule-I (VCAM) and intracellular adhesion molecule-I (ICAM) were coupled to the nps surface, while the anti-angiogenic peptide molecule CBO-P11 was encapsulated inside the carriers.

They obtained small-size nanocarriers of 70-80 nm with good spherical morphology, which did not show any hemolytic effect either in pig or human erythrocytes and any cytotoxic effect on HUVEC cells.

Ab-functionalized nanoparticles were tested both, in vitro and in vivo. They showed high cell-membrane affinity with inflamed cell-culture models and the ability to effectively detect and attach newly formed angiogenic blood vessels in animal models. Additionally, they showed the ability of the encapsulated peptide drug to reduce the proliferation of inflamed HUVECs in vitro, compared to the free drug.

This recent study demonstrates the potential of PURs as versatile nanocarriers to achieve both, drug release and targeting.

PURs with proper hydrophilic/hydrophobic balance have also been proposed for the preparation of self-assembly micelles, bearing pH- or temperature-sensitive sites.[113]

For instance, Wang and co-workers prepared temperature and pH-sensitive PUR micelles for the controlled release on doxorubicin (DOX), a DNA-alkylating agent with potent tumor suppressive activity.[114] They

investigated the use of both, aromatic and aliphatic diisocyanates, and 2 different diols, in order to modulate the hydrophilic/hydrophobic balance of the polymers and, thus, their pH and temperature responsiveness. In details hexamethylenediisocyanate (HDI) and 4,4'-diphenylmethane diisocyanate (MDI) were used as diisocyanates, while bis-1,4-(hydroxyethyl)piperazine, N-methyldiethanolamine (MDEA) and N-butyldiethanolamine (BDEA) were selected as polyols. DOX-loaded micelles were prepared by dialysis method against ultra-pure water, after separately dissolving the polymer and the drug in DMF, followed by drop wise addition of triethylamine. MDI-based PURs showed high cytotoxicity on HUVEc cells even at low polymer concentrations (40% decrease in cell viability at 0.1 mg/ml), which was ascribed to the presence of the aromatic chain extender.

Aliphatic diisocyanate-based polymers, showing no signs of cytotoxicity up to 2.5 mg/ml, were thus selected for further DOX encapsulation and release studies. PURs based on HDI-MDEA and HDI-BDEA showed good DOX encapsulation, but a large, 2-fold increase in particles size was observed for drug-loaded micelles. DOX loading was dependent on the hydrophobicity of the macrodiol. The enhanced hydrophobic interactions between BDEA and the drug, resulted in higher encapsulation efficiency and more prolonged release, compared to HDI-MDEA PUR. Micelles prepared with both PURs showed a pH and a temperature-dependant DOX release. Drug release was accelerated at pH 4, compared to pH 7.4, due to the protonation of the amino groups in the PUR structure which led to the repulsion between charged groups, thus favoring DOX diffusion. Release was also accelerated upon temperature increase from 37 °C to 50 °C.DOX-loaded HDI-MDEA PUR micelles were also internalized to a larger extent by Huh-7 hepatocarcinoma cells compared to the free drug. Nevertheless encapsulated DOX did not show a significant cytotoxic activity on the tested cell line, while free DOX caused a 60 % reduction in Huh-7 cells viability under the same testing conditions.

Our group has recently demonstrated that preformed polyester-urethanes, based on poly(ε-caprolactone) diol, 1,6 Hexamethylene diisocyanate and aliphatic chain extenders, Ciclohexanedimethanol and n-BOC serinol, are optimal candidates for the encapsulation and release of the hydrophobic anticancer drug Paclitaxel (PX).[100] We have shown that PUR nps can be prepared by both, Nanoprecipitation and Water-in-Oil emulsion methods. Compared to widely applied polyesters, such as PLGA, PLA and PCL, PURs showed comparable, size distribution, at <200 nm, morphology, cytotoxicity and internalization by model cancer cells. Additionally, when prepared by the nanoprecipitation method, they displayed significantly higher encapsulation efficiency (at 80%, compared to nearly 30% of commercial polymers), and a longer and more controlled release profile of the encapsulated drug. This feature is particularly interesting, as PX is one of the most potent anti-cancer drugs but has limited applicability due the high systemic toxicity and rapid clearance.

We have also proposed biocompatible PURs to prepare Ab-coated PX-loaded nanoparticles for the active targeting of breast cancer cells. The Ab (Herceptin), which is designed to specifically target the HER-2 over-expressing cancer cells, was introduced on the nps surface through electrostatic interactions between negatively-charged nps and positively-charged Ab.

When tested in vitro on HER-2 over-expressing breast cancer cells (SKBR-3), PUR nps showed a higher internalization potential and a better ability to induce cytotoxicity, thus retaining and enhancing drug activity, compared to commercial polyesters. Additionally, the introduction of the n-BOC serinol chain extender, which bears BOC-protected amino groups, offers the possibility to introduce specific functionalities on the nps surface for stable coupling of targeting molecules.

Although very few studies have been conducted on PUR nanocarriers for drug release so far, results summarized in this paragraph evidence the wide potential of this versatile class of biomaterials to introduce breakthrough results in nanomedicine.

5. Conclusion

The term PUR usually defines a class of polymers, which have in common the presence of urethane bonds connecting macromer blocks which can have varying chemical composition. Therefore it is more precise to consider the PUR approach as a synthetic strategy to put together, with a simple and controllable bottom-up

chemistry, several functional building blocks which constitute the ideal "ingredients" for an engineered material able to open the way towards new technological solutions in the biomedical field. Biomimicry, self-assembly, stimuli sensitive behavior, tailored degradation can now all be achieved in a single macromolecular system designed at the nano/molecular scale to satisfy all the requirements of the most challenging areas of biomedical research: tissue reconstruction and cancer treatment. Further research is needed, especially in the development of suitable in vitro models, to render this promising material platform fully viable for the development of products in bio- and nanomedicine which are ready for the market.

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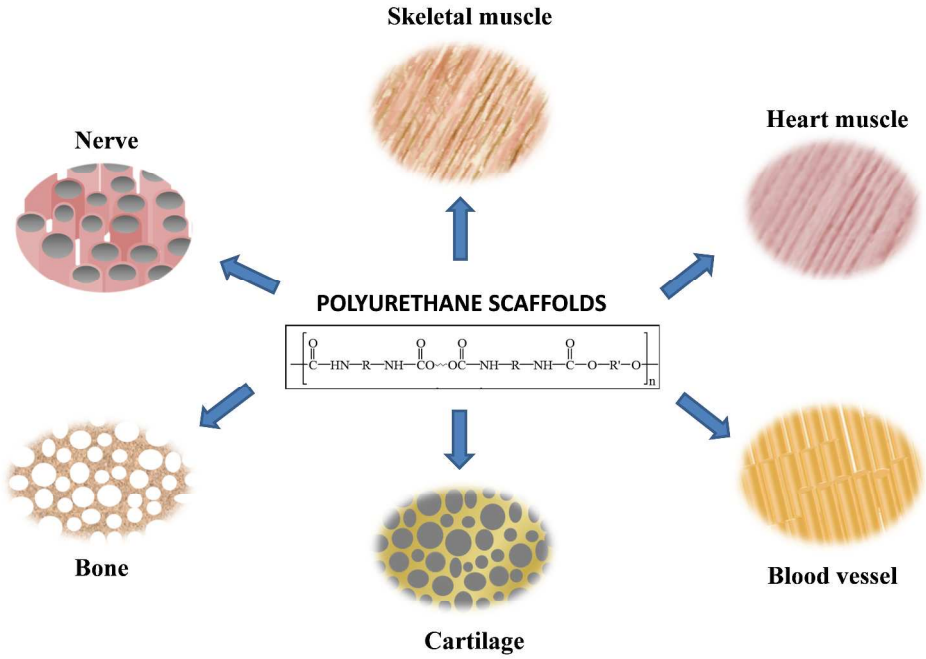

Figure 1: Polyurethane scaffolds were studied in the replacement of different tissues, represented in this scheme
1587x1190mm (96 x 96 DPI)

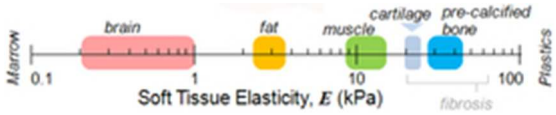

Figure 2. Soft tissue elastic modulus as a reference for the design of suitable scaffolds. Reprinted with permission.3 Copywright 2009, The American Association for the Advancement of Science.
23x4mm (300 x 300 DPI)

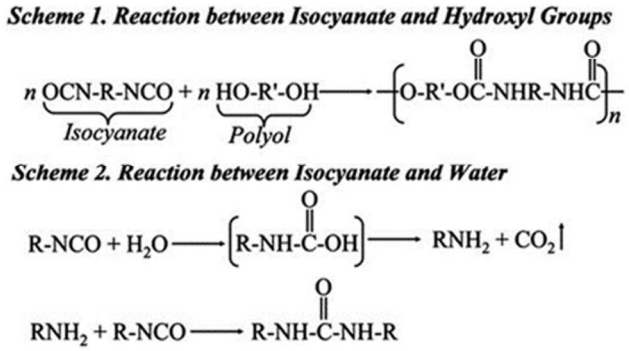

Figure 3. Schematic representation of reaction between isocyanate groups with hydroxyl groups in polyols (Scheme 1) and water (Scheme 2). Reprinted with permission.39 Copyright © 2012 Wiley Periodicals, Inc.

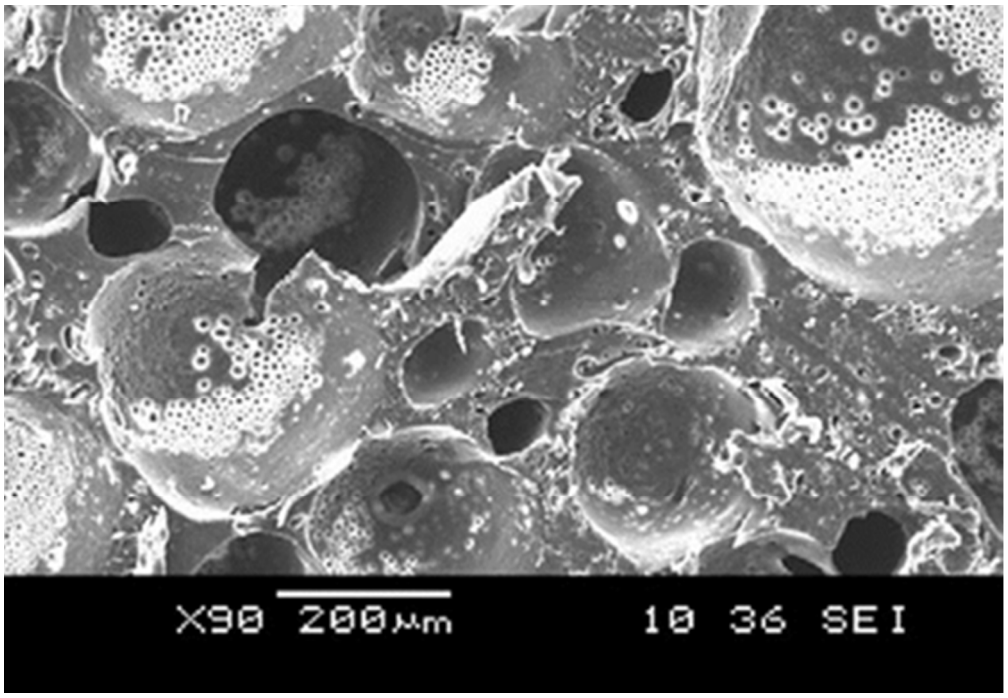

Figure 4. Fractured section of a PU/HA (1% HA) adhesive material. Reprinted with permission.39 Copyright © 2012 Wiley Periodicals, Inc

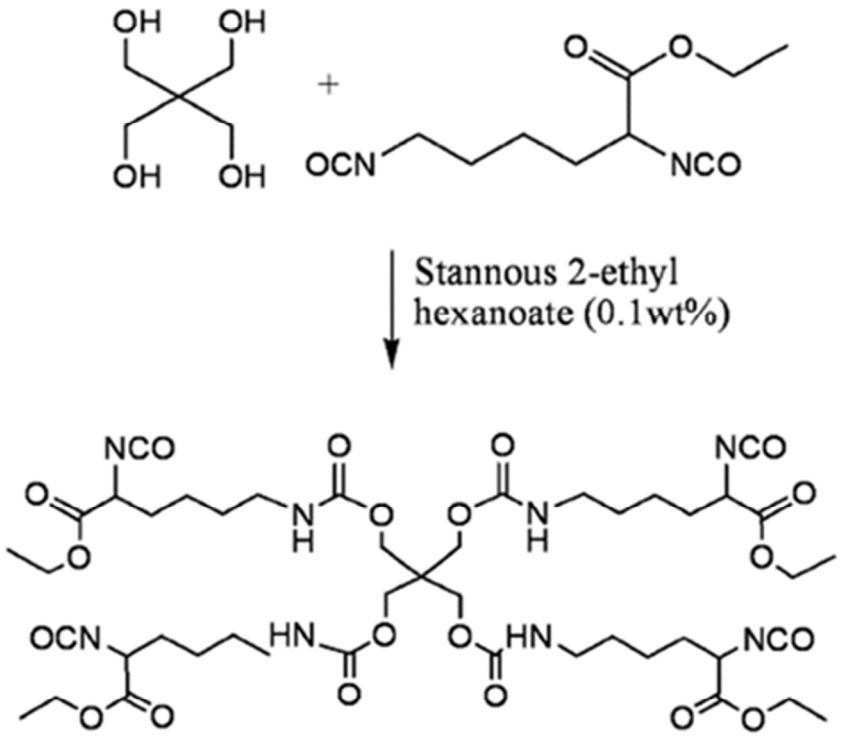

Figure 5. Schematic representation of the synthesis of prepolymer A, based on pentaerythritol end capped with ethyl lysine diisocyanate. Reprinted with permission.46 Copyright 2007, Elsevier

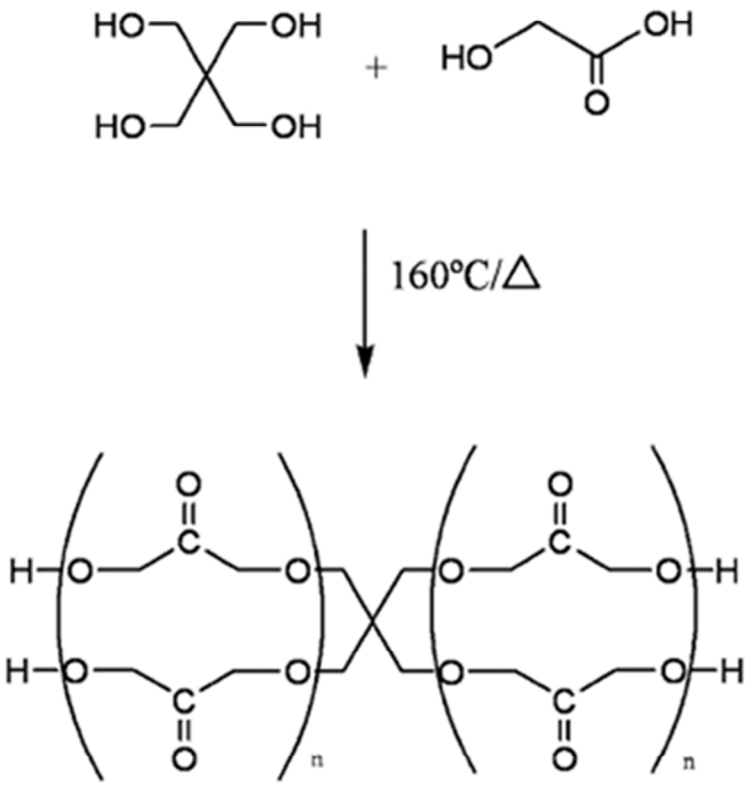

Figure 6. Schematic representation of the synthesis of prepolymer B: condensation reaction. Reprinted with permission.46 Copyright 2007, Elsevier.

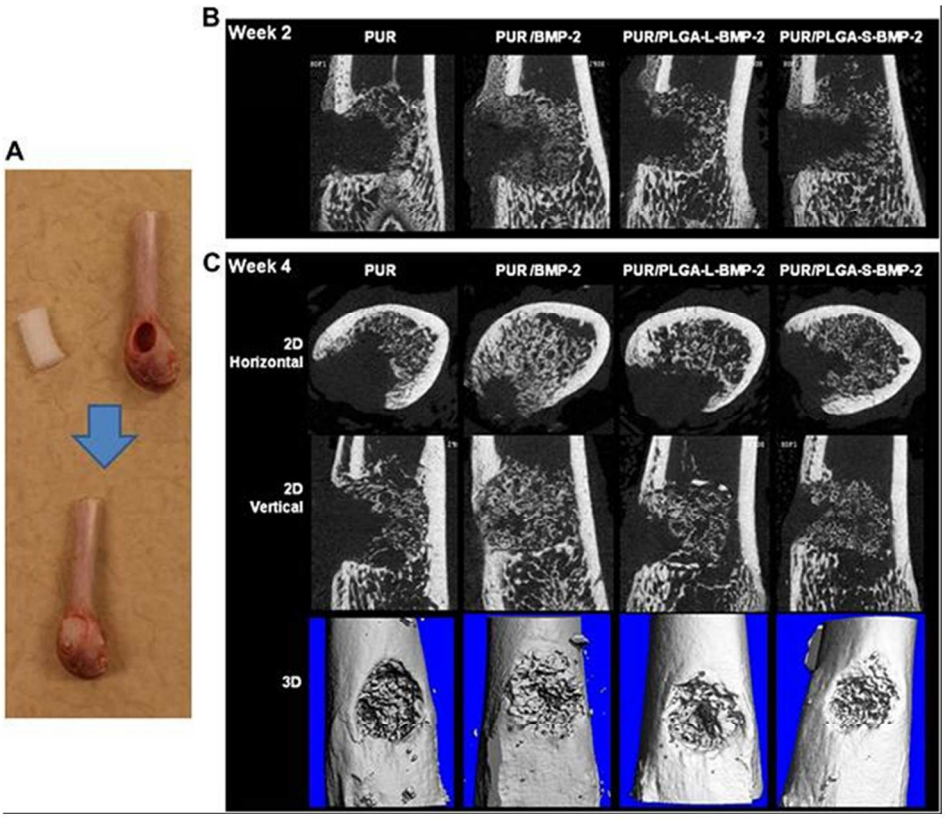

Figure 7. Representation of the implant intervention (femoral plug defects) (A) and results of in vivo tests in rat femoral plug defects obtained by micro-computed tomography (mCT) analysis after 2 and 4 weeks post-implantation (B: 2 weeks; C: 4 weeks). Reprinted with permission.48 Copywright 2014, Elsevier

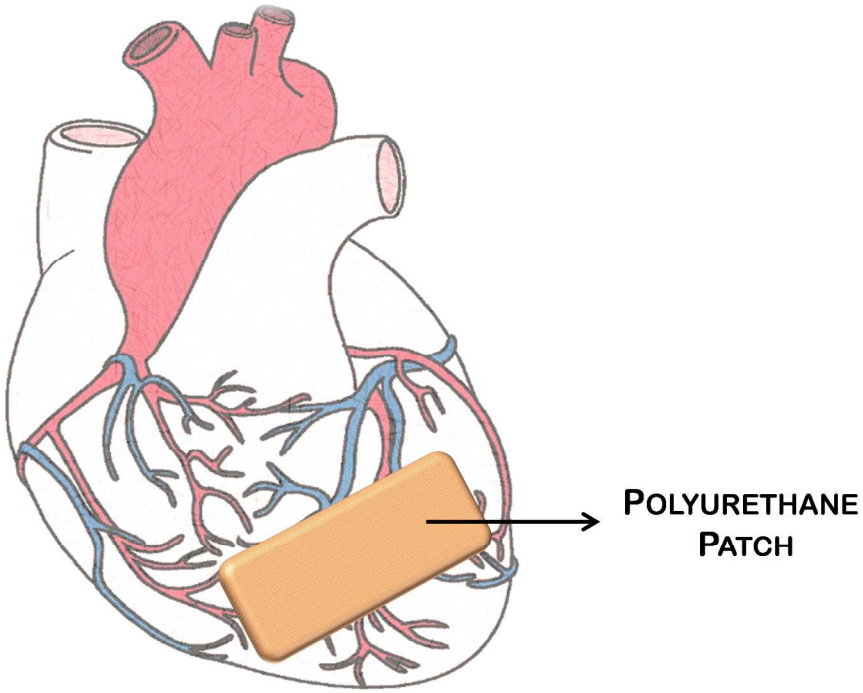

Figure 8. Engineered cardiovascular tissue graft, or "patch", applied in the infracted area and aim to support tissue regeneration

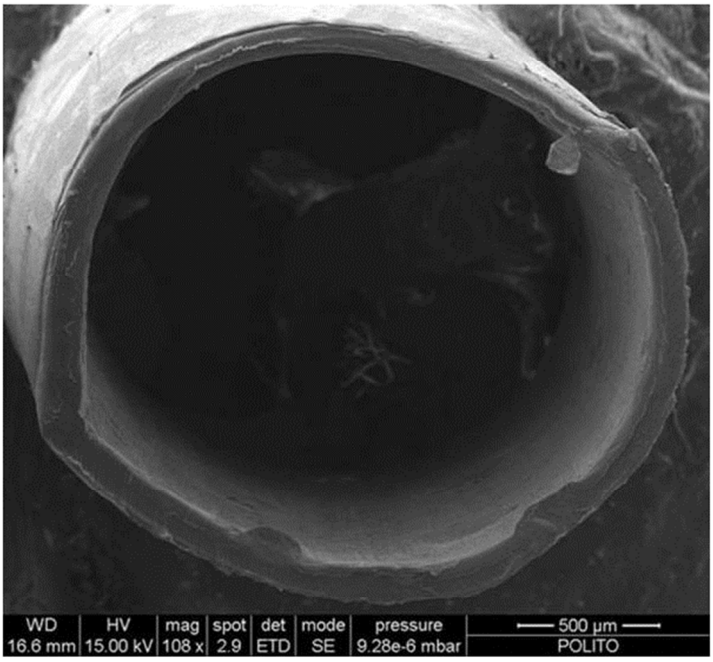

Figure 9. SEM images of a fractured section of a PU guide. Nerve guide was produced by melt-extrusion from a biocompatible novel polymer: a synthesized poly(ester urethane) having PCL as macrodiol and two commercial molecules as chain extender and linker (CDM and HDI, respectively).