Custom-made poly(urethane)s as ingredients in printing techniques towards personalized medicine.

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***Abstract*—In this contribution we report on the huge potential of customized poly(urethane)s (PURs) as raw materials to 3D print matrices for personalized medicine. During the last decade, we have focused our research on the design of PURs for both melt-extrusion and bioprinting techniques, demonstrating that polymers which belong to this family effectively represent a valuable alternative towards the definition of patient-specific therapeutic approaches.**

Keywords—poly(urethane)s, additive manufacturing, bioinks, melt-extrusion additive manufacturing, personalized medicine

I. Introduction

The term PUR defines a large family of polymers with an enormous diversity of chemical compositions and properties. This relevant versatility has allowed PURs to find widespread application in different technological fields (e.g., painting automotive, clothing, construction). Starting from the 1970s, PURs have also attracted the interest of biomedical companies that saw a great promise in their high mechanical flexibility, combined with their high tear strength [1]. Indeed, their block-copolymer structure endows them with a wide range of versatility in terms of physico-chemical properties, blood/ tissue compatibility and biodegradation. During this half a century, PURs have been designed in the form of biostable and biodegradable thermoplastic polymers targeting both long- and short-term applications, such as vascular grafts and scaffolds for tissue engineering/regenerative medicine (TERM). During the last decades, water-soluble PURs have also been developed as constituents of injectable formulations for drug delivery or bioinks for 3D bioprinting. The possibility to achieve so many different properties relies on the LEGO structure of PURs that typically comprises three building blocks: a diisocyanate, a macrodiol and a chain extender. Indeed, because of these three degrees of freedom, a virtually infinite number of different PURs can be designed. Moving from this knowledge, over the last 10 years we have grounded our research on the design of PUR biomaterials and their use as ingredients in the set-up of new therapeutic patient-specific approaches exploiting additive manufacturing technologies.

II. Materials & Methods

A. Poly(urethane) synthesis

A two-step polymerization procedure was conducted to synthesize PURs based on Poloxamer® 407 (P407) or poly(ε-caprolactone) (PCL), resulting in amphiphilic water-soluble and thermoplastic PURs, respectively [2,3]. Different chain extenders were exploited for PUR synthesis, ranging between commercial diols and diamines. An aliphatic diisocyanate was used to avoid cytotoxicity risks associated with their degradation products [1]. Successful PUR synthesis was proved through Infrared (IR) spectroscopy and Size Exclusion Chromatography (SEC).

B. Thermoplastic PURs to 3D print scaffolds

In view of its use in melt-extrusion additive manufacturing (AM), a selected PUR [2] was characterized for its thermal properties through ThermoGravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and Rheology. The PUR was then microfabricated into bi-layered scaffolds with a 0°/90° lay-down pattern through a customized melt-extrusion AM instrument developed by Università Campus Bio-Medico di Roma [4]. Printed structures were also surface functionalized with Laminin 1 (LN1) through plasma technology to better mimic the native myocardial milieu [5]. In detail, the matrices were subjected to plasma treatment for acrylic acid grafting/polymerization and finally grafted with LN1 through carbodiimide chemistry. *In vivo* testing was performed through subcutaneous implantation in mice.

C. Amphiphilic water-soluble PURs as bioink constituents

The capability of PUR aqueous solutions to gel at 37°C with improved kinetics compared to P407-based samples was first assessed [3]. Then, gel dissolution profile was tested in a physiological mimicking milieu (i.e., at pH 7.4 and 37°C). Finally, their potential as bioinks for 3D bioprinting was evaluated, together with their capability to keep their shape over time. In parallel, supramolecular (SM) hydrogels were designed by blending PURs with cyclodextrins (CDs) to further tune gel mechanical, self-healing and shear-thinning properties [6]. Additionally, by exploiting the possibility to expose photo-curable groups along PUR backbone through a proper selection of their building blocks, photo-responsive PURs are currently being explored as potential ingredients of bioinks allowing a further stabilization of 3D bioprinted structures by UV or Vis light irradiation [7].

III. Results and Discussion

A. PUR synthesis

The successful synthesis of PCL- and P407-based PURs was proved by SEC that highlighted the achievement of high molecular weight polymers which IR spectra exhibited the typical absorbance peaks of the newly formed urethane bonds.

MIUR, Italian Ministry of Education University and Research

B. *Thermoplastic PURs to 3D print scaffolds*

An elastomeric PCL-based PUR containing lysine ethyl ester as chain extender was selected as raw material to microfabricate multilayered scaffolds for cardiac TERM. Rheological and DSC characterizations proved that the PUR was in the molten state at temperatures higher than 155°C, meanwhile isothermal TGA demonstrated the absence of thermal degradation over time [4]. Bi-layered scaffolds finely reproducing the CAD design were successfully produced (Figure 1) and proved to support cardiac progenitor cell (CPC) adhesion and spreading. Differently, CPC proliferation and differentiation towards the cardiac, endothelial and smooth muscle cell lineages were successfully achieved upon scaffold surface functionalization with LN1 [5]. In addition, scaffold subcutaneous implantation in mice demonstrated their cytocompatibility and integration into the host tissue [5].

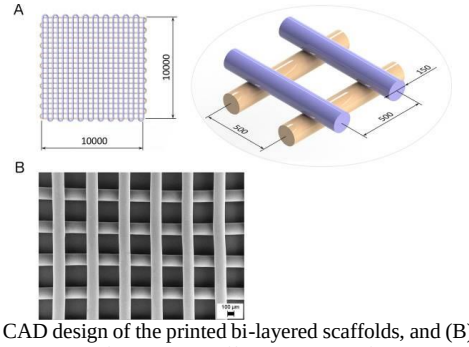

Fig. 1. (A) CAD design of the printed bi-layered scaffolds, and (B) Scanning Electron Micrograph of a printed scaffold. Reprinted from [5].

C. *Amphiphilic water-soluble PURs as bioink constituents*

The characteristic fast dissolution rate in aqueous media and the weak mechanical properties of P407 gels usually limit their biomedical application. The introduction of P407 into PUR backbone leading to high molecular weight PURs effectively overcome the previously discussed drawbacks. Indeed, PUR-based aqueous solutions exhibited thermal gelation at lower concentrations compared to P407-based ones (critical gelation concentration:6% w/v *vs.* 18% w/v). In addition, PUR hydrogels exhibited higher gel strength (G' at least 4-fold higher), faster gelation kinetics (<5min *vs.* 15-30min) and improved stability in aqueous media (weeks *vs.* few days) compared to their P407-based counterpart. In addition, they showed improved capability to keep their shape (PUR gels exhibited a compact and solid-like structure) and quickly formed upon injection [3]. Such properties were successfully exploited to 3D bioprint multilayered structures with good resolution (Figure 2). More recently in order to further improve the mechanical, self-healing and shear-thinning properties of our PUR hydrogel platform as well as to provide them with a bioartificial composition, SM gels were designed by blending PURs and CDs at 1-9% w/V and 9-10% w/V concentration, respectively [6]. Although we developed bioinks with improved mechanical properties and residence time in aqueous media compared to P407 gels, the physical nature of such gels still did not allow their use for long-term applications. In order to overcome this issue, we are currently exploiting the wide availability of building blocks for PUR synthesis to introduce pendant photo-responsive moieties along their backbone. For instance, we have recently reported on the synthesis of a P407-based PUR bearing pendant thiol groups which could be exploited to design thermo- and photo-sensitive bioinks. Thermo-responsivess will allow the bioinks to be 3D bioprinted in cell friendly conditions and will provide the resulting structures with a primary stability which will be then further improved through photocuring [7].

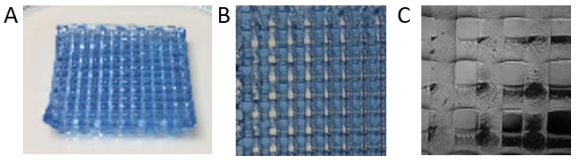

Fig. 2. (A) Photo of a PUR-based multi-layered 3D bioprinted matrix; (B) and (C) photo and optical microscope images of a bi-layered structure.

IV. Conclusion

During the last decade we successfully provided proof of concept results on the huge potential of PURs as ingredients for AM technologies. Their customized nature together with the enormous versatility of AM instruments will allow in the future to maximize the outcomes of personalized medicine. In this regard, automation and instrument customization will play an essential role, allowing an easy printing of *ad-hoc* designed biomaterials, such as PURs themselves and composites (e.g., printing starting from pellets instead of filaments). In addition, PUR advanced and versatile chemistry as well as their highly programmable properties will be also exploited to develop raw materials for 4D printing.

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References

[1] P. Vermette, H. J. Griesser, G. Laroche, and R. Guidon, Biomedical Applications of Polyurethanes, Landes Bioscience, 2001.

[2] S. Sartori, M. Boffito, P. Serafini, A. Caporale, A. Silvestri, E. Bernardi, et al., "Synthesis and structure-propert relationship of polyester-urethanes and their evaluation for the regeneration of contractile tissues", React. Funct. Polym., vol. 73, pp. 1366-1376, 2013.

[3] M. Boffito, E. Gioffredi, V. Chiono, S. Calzone, E. Ranzato, S. Martinotti, and G. Ciardelli, "Novel polyurethane-based thermosensitive hydrogels as drug release and tissue engineering platforms: design and in vitro characterization", Polym. Int., vol. 65, pp. 756-769, 2016.

[4] V. Chiono, P. Mozetic, M. Boffito, S. Sartori, E. Gioffredi, A. Silvestri, et al., "Polyurethane-based scaffolds for myocardial tissue engineering", Interface Focus, Vol. 4, 20130045, 2014.

[5] M. Boffito, F. Di Meglio, P. Mozetic, S. M. Giannitelli, I. Carmagnola, C. Castaldo, et al., "Surface functionalization of polyurethane scaffold mimicking the myocardial microenvironment to support cardiac primitive cells", PLoS ONE, Vol. 13, e0199896, 2018.

[6] A. Torchio, M. Boffito, A. Gallina, M. Lavella, C. Cassino, and G. Ciardelli, "Supramolecular hydrogels based on custom-made poly(ether urethane)s and cyclodextrins as potential drug delivery vehicles: desing and characterization", J. Mater. Chem. B, Vol. 8, pp. 7696-7712, 2020.

[7] R. Laurano, C. Cassino, G. Ciardelli, V. Chiono, and M. Boffito, "Polyurethane-based thiomers: a new multifunctional copolymer platform for biomedical applications", React. Funct. Polym., Vol. 146, 104413, 2020.