Abstract

The repair of critical-sized tissue defects remains a significant challenge in regenerative medicine because damaged tissues often lack the intrinsic ability to fully restore their structure and function. Tissue engineering (TE) has emerged as a promising approach to address this limitation through the use of biomaterial scaffolds that provide temporary mechanical support while simultaneously guiding cellular activities necessary for tissue regeneration. An ideal scaffold should mimic the structural and functional characteristics of the native extracellular matrix by possessing a highly interconnected porous architecture that facilitates cell infiltration, vascularisation, nutrient diffusion, and metabolic waste transport. In addition, the scaffold must exhibit adequate mechanical integrity to withstand physiological conditions and degrade in a controlled manner as newly formed tissue gradually replaces the implanted material. Therefore, achieving an optimal balance between porosity, mechanical stability, biological performance, and biodegradability remains a major challenge in the development of next-generation tissue engineering scaffolds. Furthermore, the incorporation of dynamic stimulus-responsive behaviour has gained increasing importance, particularly for minimally invasive surgical procedures and large soft-tissue defects, where scaffolds are required to undergo temporary deformation or compression during implantation and subsequently recover their original shape under physiological conditions. Among the various scaffolding techniques, emulsion templating has attracted significant attention because of its ability to generate highly porous and interconnected architectures with tunable structural properties. This method generally involves the preparation of an emulsion containing two immiscible liquid phases, where one phase is dispersed within the other. Subsequent solidification of the continuous phase and removal of the dispersed phase results in the formation of a porous scaffold. Using this approach, scaffolds with porosities of up to 99% and controllable pore morphologies can be achieved. Furthermore, this technique can be merged with 3D printing to generate customised hierarchical porous scaffolds.

Emulsion-templated scaffolds have been fabricated using a broad range of synthetic and natural polymers. However, we are particularly interested in the development of a poly(caprolactone) (PCL)-based emulsion-templated system because of its inherent tunability, tissue-like mechanical properties, and relatively high degradation time of 2-3 years. But emulsion templating of PCL remains challenging due to the inherently high viscosity of PCL, which hinders the efficient mixing and stabilisation of the emulsion phases. On the other hand, 3D printing of emulsion-templated PCL formulations is also challenging without significantly high

Pickering stabiliser loading. So, this requires perfect engineering of the printability of emulsion ink. Ideally, we have to engineer a dual network-based system that behaves like a viscoelastic spring-dashpot under high shear extrusion during 3D printing, where the brittle network would dissipate energy by the fracture of its crosslinks and the flexible network remained intact and typically allowed the emulsion to recover from strain.

Herein, aqueous high internal phase emulsion (HIPE) templating was employed to generate microporosity within the PCL matrix. The resulting HIPE ink was subsequently 3D printed to introduce interconnected macroporosity between the printed strands, thereby yielding a hierarchically porous scaffold comprising both micro- and macroporous architectures. Either high-molecular-weight PCL, methacrylate-functionalised PCL, or a combination of both was used, depending on the requirements. Hydrophobically modified nanoclay Cloisite 30B was used as Pickering stabiliser, and aqueous solution of polyvinyl alcohol (PVA) was taken as the disperse phase. Prior to the printing, the effect of PCL concentration, Cloisite 30B concentration and volume of diluent was optimised by analysing HIPE morphology and printability. This optimisation acted as the foundation of this research. The principal outcome of this fundamental study was the significant improvement in scaffold printing resolution achieved at a substantially lower nanoparticle loading (1.5% w/v), representing up to a 10-fold reduction compared to previous studies that typically employed at least 15% nanoparticle loading, while simultaneously preserving the flexibility of the fabricated scaffolds. The prepared scaffolds demonstrated exceptionally high porosity and favourable biomineralisation capability; however, the interconnected porous network resulted in insufficient mechanical strength, poor bioactivity and rapid degradation. The next chapter was dedicated to improving the mentioned properties. To improve structural stability while retaining printability and porosity, a semi-IPN strategy was subsequently introduced by combining a flexible polymeric matrix with a reinforcing secondary network. This approach enabled the formation of superior printable HIPE inks with excellent near-ideal shape fidelity and enhanced mechanical performance. In addition, inorganic bioactivation and herbal functionalisation improved cellular response, osteogenic activity, and oxidative stress resistance. But this scaffold lacked dynamic responsive behaviour.

To further introduce dynamic responsive behaviour into the scaffold system, thermo-responsive vitrimeric PCL networks based on dynamic imine exchange chemistry were developed. These materials exhibited self-healing behaviour, shape-memory capability, and reversible deformation under physiological conditions. The influence of phase morphology on

thermomechanical properties and surface characteristics was systematically investigated, revealing that co-continuous architectures provided superior shape recovery and fixation performance. Although these vitrimeric systems demonstrated excellent adaptability, their dense structure limited effective cellular infiltration. To overcome this limitation, the final part of the thesis focused on integrating hierarchical porosity with responsive vitrimeric behaviour through the development of a solvent-free DES-mediated Pickering HIPE platform for 4D printing. A camphor-menthol DES was employed as a sustainable diluent to dissolve PCL and stabilise printable emulsion formulations without the use of volatile chlorinated solvents. The resulting scaffolds exhibited interconnected hierarchical porosity, tunable mechanical properties, efficient shape-memory response at body temperature, and enhanced biological performance. The incorporation of vitrimer chemistry within the semi-IPN porous architecture further enabled stress relaxation, improved toughness, and controllable surface topography while preserving reversible actuation behaviour.

Overall, this study demonstrates the development of a highly tunable, chlorinated organic solvent-free, hierarchically porous, thermoresponsive PCL scaffold with significant potential for tissue engineering applications.