3D Printed Highly Elastic Scaffolds Reinforced by Electrospun Fibers for Uterine Tissue Engineering

Shangsi Chen [1], Jizhuo Chen [1], Liwu Zheng [2], Min Wang [1,*]
[1] Department of Mechanical Engineering, [2] Faculty of Dentistry, The University of Hong Kong, Pokfulam Road, Hong Kong
Email: memwang@hku.hk.

Introduction: Disease or injury of the uterus can cause infertility. Tissue engineering strategies which aim to combine biomaterials with stem cells and biomolecules in 3D scaffolds have proved to be an effective way to treat damaged body tissues [Ovsianikov A, *et al*., *Trends in Biotechnology*, 2018, 36(4): 348-357]. Furthermore, 3D printing has become popular for producing 3D tissue engineering scaffolds due to its distinctive advantages, including patient-customization, interconnected pores for nutrient and oxygen exchange, and spatiotemporal control of drug/biomolecule delivery [Do AV, *et al*., *Advanced Healthcare Materials*, 2015, 4(12): 1742-1762]. Although 3D printing has been extensively used in various tissue regeneration, it is rare to use it in uterine tissue engineering [Ji W, *et al*., *Acta Biomaterialia*, 2020, 116: 268-284]. In addition, the uterus is highly elastic. Previous 3D scaffolds for uterine regeneration lack high elasticity. Therefore, in this study, fabricating highly elastic scaffolds reinforced by electrospun fibers via 3D printing was investigated. The scaffolds possessed superior mechanical properties than human uterus and exhibited controlled and sustained estradiol (E2) release for cell regulation.

Methods: (1) Fabrication of TPU/PDA@E2 electrospun fibers: Polydopamine particles loaded with E2 (PDA@E2) (with the dopamine hydrochloride to E2 ratio at 1:0.3) were synthesized in a weak alkaline solution (10mM Tris solution, pH8.5). E2 is poorly water soluble. Incorporation of E2 in PDA particles could promote E2 solubility and bioavailability. Subsequently, PDA@E2 particles were homogenously distributed in TPU solution to fabricate electrospun fibers. Electrospinning was conducted at 20kV applied voltage and 2.0mL/h solution feeding rate. (2) Fabrication of uterine tissue engineering scaffolds: Electrospun TPU/PDA@E2 fibers were homogenously dispersed in poly(L-lactide-co-trimethylene carbonate) (PLLA-TMC, LA:TMC ratio at 5:5) solution. The mixture was transferred into printing cartridge for 3D printing. 3D printed scaffolds were characterized by using SEM and TEM, and their mechanical properties were assessed via tensile tests. *In vitro* E2 release behaviour of 3D printed scaffolds was studied. *In vitro* biological performance of the scaffolds was also investigated.

Results: The incorporation of E2 in PDA particles could promote E2 solubility and bioavailability. According to SEM and TEM observations, PDA@E2 particles had a spherical shape with diameters of 800 - 1,000 nm (Fig.1a). The encapsulation of E2 did not affect the PDA particle shape or morphology. Additionally, TPU fibers with different PDA@E2 particle concentrations were successfully electrospun. Based on tensile test results, fibers having PDA@E2 particles at 40 wt.% was used in subsequent studies. PDA@E2 particles were generally well-distributed in TPU fibers even though there were some aggregated beads (Fig.1b). Subsequently, electrospun TPU fibers were mixed with a PLLA-TMC solution for 3D printing. With the addition of TPU fibers, the printability of the PLLA-TMC solution improved. Moreover, TPU/PDA@E2 fibers were homogeneously distributed in PLLA-TMC struts of printed scaffolds (Fig.1c). Because of the reinforcement of TPU/PDA@E2 fibers, the mechanical properties of printed scaffolds were enhanced. The tensile strength of pure PLLA-TMC scaffold was about 500KPa, which is lower than that of uterus. But with TPU/PDA@E2 fiber addition at 30 wt.%, the tensile strength was increased to 2.6 MPa, which is comparable to native uterus (Fig.2a). Furthermore, scaffolds reinforced by TPU/PDA@E2 fibers exhibited significantly enhanced cell behavior. Bone marrow-derived mesenchymal stem cells (BMSCs) were well-spread on these scaffolds, and the cell proliferation rate was higher than on pure PLLA-TMC scaffolds (Figs.2b to 2d).

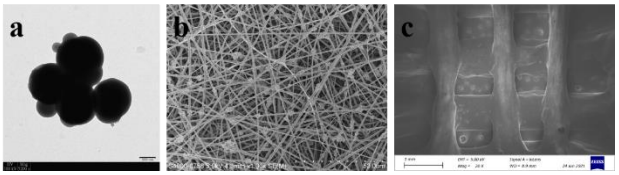

Fig. 1. (a) TEM image of PDA@E2 particles, (b) SEM image of electrospun TPU/PDA@E2 fibers, (c) SEM image of 3D printed fiber-reinforced PLLA-TMC scaffold.

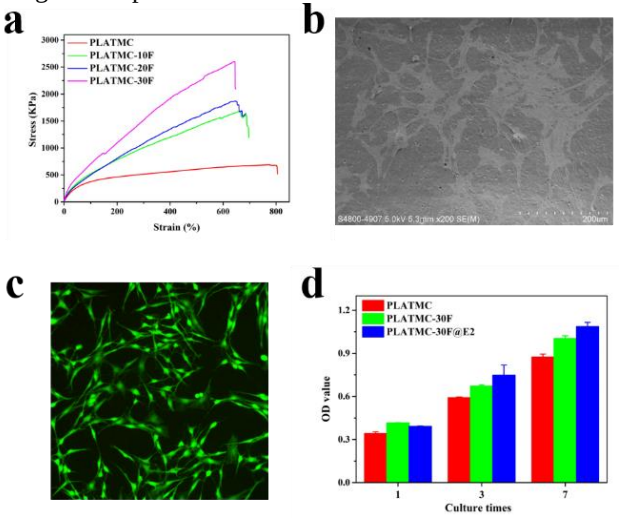

Fig. 2. (a) Tensile curves of scaffolds, (b) SEM image showing BMSC cell attachment, (c) image indicating live BMSCs, (d) BMSC proliferation rates for different 3D printed non-reinforced and reinforced scaffolds.

Conclusions: PDA@E2 particles were well-distributed in electrospun TPU fibers, and the fibers were well-dispersed in PLLA-TMC struts of 3D printed scaffolds. The incorporated fibers reinforced the scaffolds. The *in vitro* biological study indicated that the printed composite scaffolds were biocompatible and could enhance cell behavior such as cell attachment and proliferation. The 3D printed scaffolds reinforced by TPU/PDA@E2 fibers are attractive structures for uterine tissue regeneration.

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