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Functional gradient scaffolds capable of dual drug spatiotemporal release

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A novel 3D bio-printing platform is integrated with a forming system that combines electrospinning and extrusion deposition to prepare drug-loaded structures.

Functional gradient scaffolds play an important role in osteochondral (i.e., cartilage) tissue engineering. This is because the scaffolds meet the essential requirement for a gradual transition of both physical and chemical properties in osteochondral tissue regeneration.[1–4] There remains, however, a further requirement for 3D composite osteochondral regeneration scaffolds with multi-scale structures that are capable of controlled release of multiple biomolecules.

At present, a composite-forming system—in which 3D bio-printing and an electrospinning system are combined—is used to fabricate hierarchical scaffolds in an extensive number of in vitro and in vivo studies.[5–8] With this method, however, it has been difficult to achieve homogeneous cell distribution within the scaffolds. To guide the regeneration of tissue structure and function, an ideal scaffold should provide molecular cues in addition to structural support, mechanical properties, and biodegradable features. During natural tissue development and regeneration, multiple biomolecules play key roles in the processes that lead to tissue formation.[9, 10] Treatments aimed at mimicking tissue regeneration could therefore benefit from the release of multiple therapeutic agents, and it has been suggested, based on the results of these previous studies, that the local release of multiple biomolecules is a promising approach for regenerative medicine.

In this work,[11] we have therefore developed a 3D bio-printing platform that is integrated with a forming system to produce scaffolds loaded with various drugs (see Figure 1). We used a self-developed 3D bio-printing platform, in which we combine extrusion deposition and multi-nozzle electrospinning, to fabricate our novel scaffold structures. In particular, we produced a hierarchical composite scaffold that consisted of gelatin/sodium alginate (SA) struts and electrospun dual-drug-loaded nanofibers (see Figure 2). We are able to obtain a

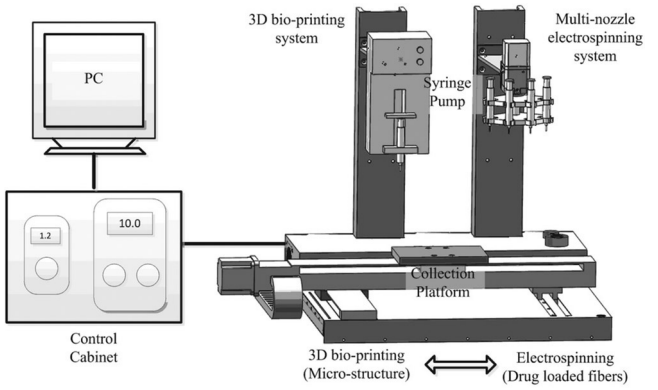

Figure 1. *The 3D bio-printing platform integrated with a forming system that combines extrusion deposition and multi-nozzle electrospinning.*

vertical gradient porosity along the thickness of our structures because the scaffolds contain gradient-changing constructs. It is thus possible to realize spatially controlled release of desferoxamine (DFO), i.e., an iron chelating agent approved by the US Food and Drug Administration. This is a co-factor for the prolyl hydroxylase enzyme that degrades the hypoxia inducible factor. It induces angiogenesis and improves fracture healing through the upregulation of vascular endothelial growth factor.[12, 13] The sustained release and delivery of DFO is therefore desirable and effective for bone healing.

For temporally controlled release of biomolecules, we developed a multi-nozzle switching electrospinning subsystem of our 3D biological printing platform. We thus produced blended and coaxial nanofibers with the use of different electrospinning processes. We incorporated gentamycin sulfate (GS) and DFO into blended electrospun polyvinyl alcohol (PVA) nanofibers and coaxial electrospun nanofibers (with PVA-DFO cores and polycaprolactone shells), respectively. GS is an anti-inflammatory drug that can significantly lower the expression level of pro-inflammatory cytokines.[14, 15] By using these two types of

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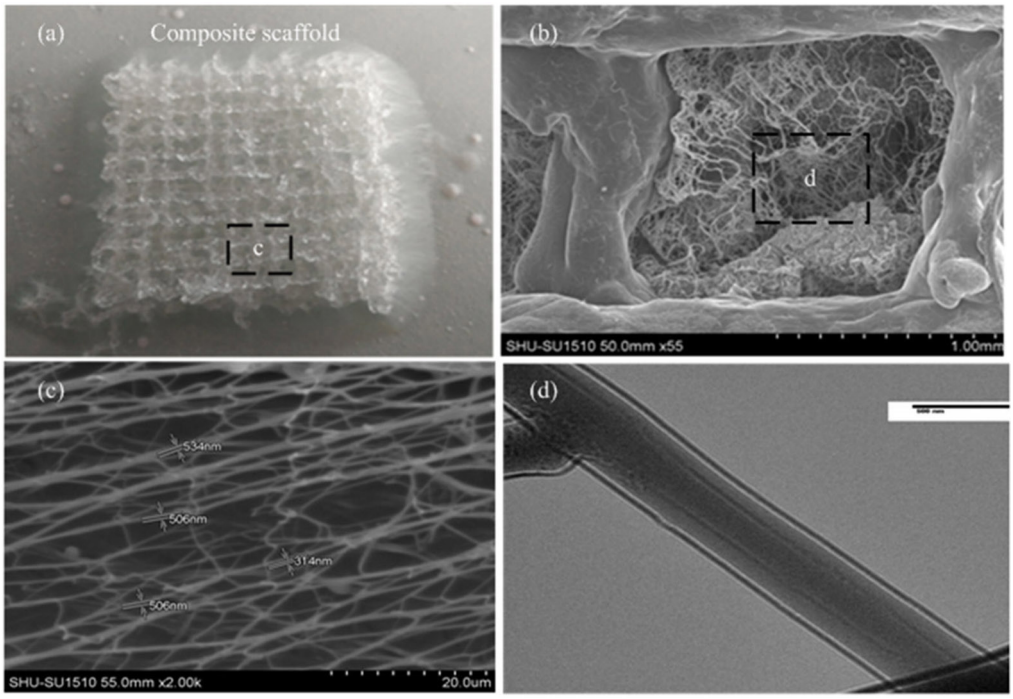

Figure 2. *(a) Photograph and (b) scanning electron microscope (SEM) image of the composite scaffold with electrospun fibers. (c) SEM image of the electrospun monolithic polyvinyl alcohol 124 (PVA) nanofibers blended with gentamycin sulfate (GS). Image is taken from the area denoted in (a). (d) Transmission electron microscope image of polycaprolactone-PVA coaxial nanofibers, from the region denoted in (b), which shows the distinguishable core-sheath structure. Scale bars in (b), (c), and (d) indicate 1mm, 10μm, and 500nm, respectively.*

nanofibers to form our composite scaffolds (with structural, compositional, and biochemical gradients), we were able to characterize the biochemical changes, as well as the degradation behavior, of the structures for up to 25 days in vitro.

The results of our 25-day time-lapse in vitro release study indicate the temporally controlled release of GS and DFO. We find (see Figure 3) that GS was released faster than DFO during the early stages and that there was a sustained release of DFO over long periods. Furthermore, we observed spatially controlled release of DFO from the vertical gradient SA scaffolds (see Figure 4). These scaffolds enabled the release of DFO in a gradient mode. Our experimental results thus demonstrate the validity of the 3D bio-printing platform integrated with the forming system, as well as the excellent properties of our scaffolds for the spatiotemporal release of multiple drugs.

An important advantage of our composite scaffold is that various release profiles can be obtained for each biomolecule. These can be achieved by using different chemistries and processes for the preparation of the electrospun fibers. In this particular study, we used the GS- and DFO-loaded scaffolds to realize versatile, temporally controlled drug release for the induction of osteogenesis. We chose these drugs because a dose of GS during the early stages of sterilization and a

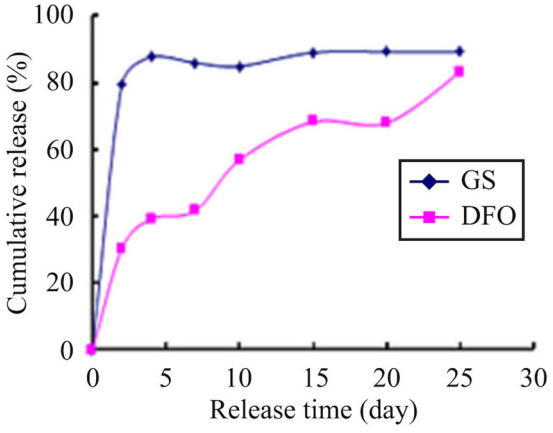

Figure 3. *Release profiles of GS and desferoxamine (DFO) from the hierarchical composite scaffold. These profiles indicate the cumulative release of DFO from coaxial fibers and of GS from blended fibers. The statistical significance of the data (*P) is < 0.05.*

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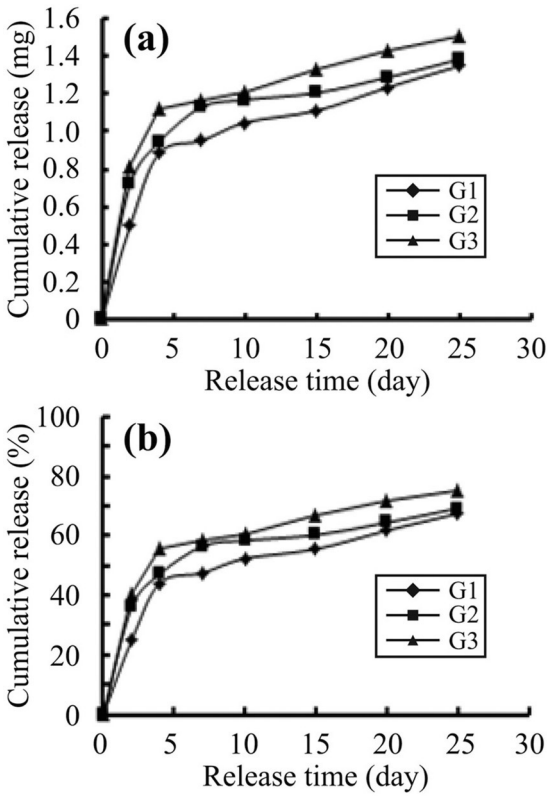

Figure 4. *Gradient release profiles of DFO from three segments (G1, G2, and G3) cut from the composite scaffold, based on (a) mass changes and (b) percent released relative to the total amount loaded per scaffold. *P < 0.05.*

sustained release of DFO are known to induce angiogenesis during osteogenic induction. In our gradient composite scaffold, DFO exhibited a decreasing gradient distribution. The amount of DFO released in the membrane also exhibited a gradient trend and spatial release. This gradient DFO release behavior could possibly satisfy the requirement for bone-cartilage tissue regeneration (i.e., by adjusting the amount of DFO or multi-growth factors via co-electrospinning). The most substantial innovation from our methodology is that different release profiles can be obtained independently for each drug, by changing the structures or properties of the struts and nanofibers separately.

In summary, we have described our 3D bio-printing process for the preparation of functional osteochondral scaffolds. We have focused on the special characteristics of the scaffolds that are integrated with gradient structures and may be loaded with multiple biomolecules. Our composite scaffold shows potential for delivery of several biomolecules, with different release profiles, over space and time. We have also demonstrated that our combination of multi-nozzle electrospinning and 3D bio-printing is a feasible method for producing functional gradient scaffolds. In the next stages of our research we will aim to develop a theoretical analysis and determine an optimization model for functional scaffolds. In addition, we will test the physiological functionality of our scaffolds through animal experiments and histological evaluations.

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