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Hierarchically engineered fibrous scaffolds for bone regeneration

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Surface properties of biomaterials play a major role in the governing of cell functionalities. It is well known that mechanical, chemical and nanotopographic cues, for example, influence cell proliferation and differentiation. Here, we present a novel coating protocol to produce hierarchically engineered fibrous scaffolds with tailorable surface characteristics, which mimic bone extracellular matrix. Based on the sol–gel method and a succession of surface treatments, hollow electrospun polylactic acid fibres were coated with a silicon–calcium–phosphate bioactive organic–inorganic glass. Compared with pure polymeric fibres that showed a completely smooth surface, the coated fibres exhibited a nanostructured topography and greater roughness. They also showed improved hydrophilic properties and a Young's modulus sixfold higher than non-coated ones, while remaining fully flexible and easy to handle. Rat mesenchymal stem cells cultured on these fibres showed great cellular spreading and interactions with the material. This protocol can be transferred to other structures and glasses, allowing the fabrication of various materials with well-defined features. This novel approach represents therefore a valuable improvement in the production of artificial matrices able to direct stem cell fate through physical and chemical interactions.

1. Introduction

Despite the considerable advances made in regenerative medicine in the last decade, the production of novel biomaterials for tissue engineering remains a challenging field of research. The complexity of the biological environment and the difficulty of producing materials that can properly interact with it make the development of such materials especially ambitious. Indeed, today's biomaterials should fulfil many requirements in order to be successfully implanted. Besides being biocompatible and having an appropriate porosity, they should also be instructive to trigger specific cellular responses. They should provide the right signals to the cells (i.e. chemical signals, topography and mechanical properties) that promote their differentiation into a particular cell lineage, and therefore stimulate the formation of new tissues [1]. As temporary templates, the degradation speed of biomaterials must be tuned to match tissue formation, with the result that they are entirely replaced by the naturally regenerated tissue. Many of the temporary tissue regeneration solutions currently developed rely on products that combine biological agents, such as cells or biomolecules. But, even though this approach has proved to be successful in some applications, it involves complications in scalable fabrication, storing and strict regulatory issues. The cost and complexity of these therapies are leading to requests for other novel and more cost-effective alternatives [2].

For bone regeneration, hybrid materials (materials composed of a mixture of inorganic components, organic components or both types of components, which usually interpenetrate on the submicrometre scale [3]) appear to be one of the most promising candidates for the design of new scaffolds, owing to their excellent bioactivity and suitable mechanical properties [4]. However,
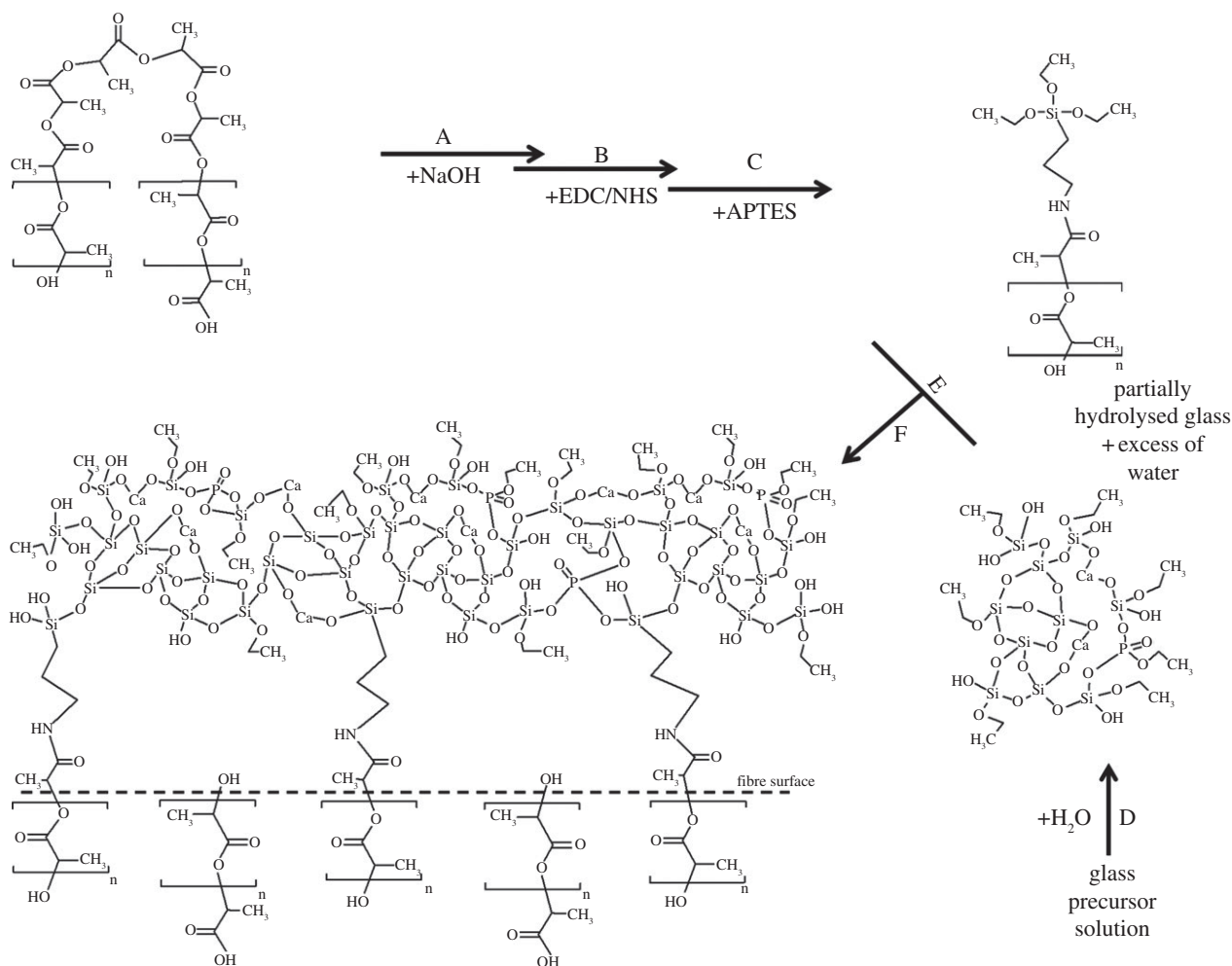
Scheme 1. Chemical coating reactions. Schematic illustration of PLA fibres coating process involving surface treatments such as (A) hydrolysis, (B) activation of reactive groups and (C) functionalization with coupling agent and (D, E and F) glass bonding.

they generally lack strong chemical interactions between their constituents, resulting in their non-homogeneous and fast degradation in body fluid. As a consequence, the degradation of the implant often mismatches the formation of the newly formed bone [4]. On the other hand, hybrid materials are usually produced by a non-tailored dispersion or mix of an inorganic phase into a synthetic polymeric matrix (bioactive glass or hydroxyapatite nanoparticles, for examples). Thus, the inorganic compound is often masked by the polymer [5]. As the cells attach preferentially to the bioactive compound rather than to the synthetic polymer [6], a previous degradation of the polymer is needed to reveal the bioactive entity underneath and achieve efficient cell adhesion. Therefore, the creation of hybrid biodegradable scaffolds that possess strong interactions (i.e. covalent bonding) between their organic and inorganic phases is required in order to have better control of the degradation process [7], as well as the entire exposure of the bioactive material at the surface to enhance cell–material interactions. All these requirements should be satisfied also in a comprehensive three-dimensional structure which mimics the fibrous structure of the extracellular matrix of natural bone.

2. Results and discussion

Accordingly, we report the fabrication of a hybrid electrospun nanofibrous scaffold obtained by a new coating protocol. Here, the nanofibrous scaffold made of poly lactic acid (PLA)

(FDA-approved for several devices and extensively used in regenerative medicine [8]) provides the flexible structural support while a sol–gel processed organic–inorganic bioactive glass (P_2O_5-CaO-SiO_2 system [9]) supplies the chemical bioactive cues. The use of this system was supported by several published studies in which good osteointegrative properties and the triggering of a proper cellular response were reported [10,11]. The gradual biodegradation of the bioactive glass (i.e. ions required to guide bone tissue repair [11,12]) will trigger the regeneration process. Electrospun fibres have been shown to faithfully mimic the fibrous components of the extracellular matrix structure of natural bone, and therefore represent a suitable basic architecture for this study [13,14]. Hollow PLA fibres were produced with a conventional electrospinning device due to the Kirkendall effect [15] and 2,2,2-trifluoroethanol as solvent. Then, fibres were subjected to a succession of surface treatments: first, controlled hydrolysis was applied to create carboxyl groups at the surface of the fibres (scheme 1A). These groups were then activated through immersion in an 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) solution (scheme 1B), and finally functionalized with the (3-aminopropyl)triethoxysilane (APTES) coupling agent (scheme 1C). In parallel, an ormoglass (organically modified glass) solution containing Si, Ca and P was partially hydrolysed in order to create a colloidal glass suspension. Hence, an organic–inorganic xerogel that contains alkoxide groups linked to the metal cations can be obtained. This suspension is then deposited on the previously APTES
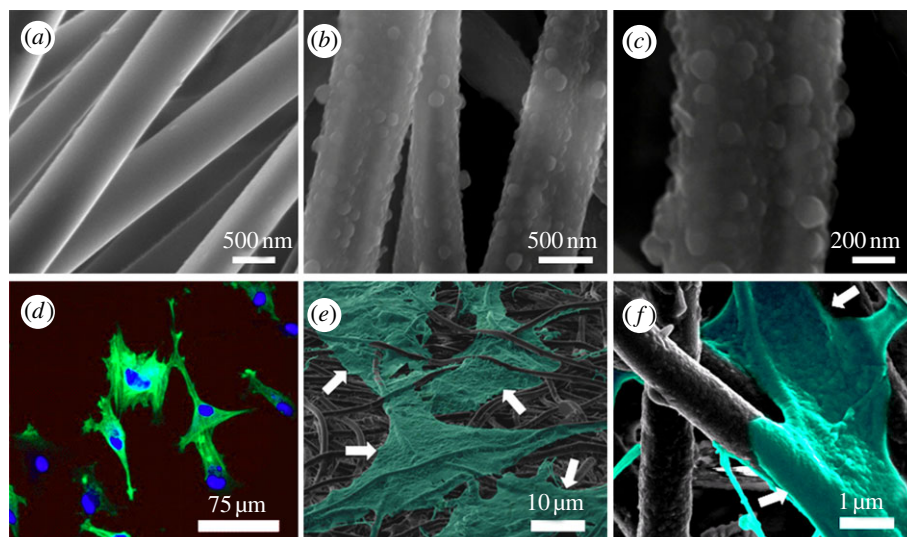
Figure 1. Fibre morphology and cell – material interactions. Topography of (*a*) pure PLA and (*b,c*) coated fibres attesting to the rough nanostructured surface of the fibres after treatments. (*d*) Confocal imaging of rMSCs cultured on coated fibres showing stress fibres inside cells. The nuclei appear as dark patches. (*e,f*) Morphology of rMSCs showing the good spreading and interactions of cells with the scaffold (arrows point the edge of the cells). (Online version in colour.)
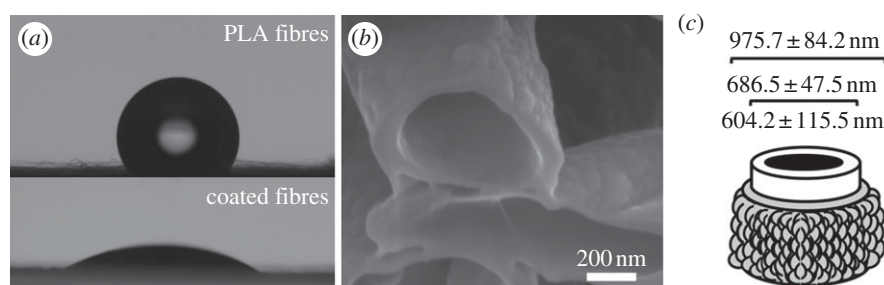

Figure 2. Fibres characteristics: (*a*) water contact angle pictures showing the excellent hydrophilicity of the coated fibres ($31.8 \pm 0.5°$) compared with the pure PLA ones ($124.3 \pm 1.2°$). (*b*) Fibre cross section obtained by focus ion beam technique showing the tubular structure of the fibres, and (*c*) detailed scheme presenting their average measured dimensions.

functionalized fibres (scheme 1D,E). Terminal ethyl groups of APTES reacted with the glass suspension through a condensation process, forming siloxane bonds and linking the glass to the polymer covalently by means of APTES (scheme 1F) [16]. Finally, to ensure that the glass particles were strongly and efficiently attached, the fibres were sonicated for 5 min.

Field emission scanning electron microscopy was used to observe the morphology and determine the thickness of the coated fibres. A rough nanostructured surface topography compared to non-treated fibres was revealed (figure 1*a*–*c*); confirmed and quantified by atomic force microscopy measurements (see electronic supplementary material, figure A). As nanophased materials have been demonstrated to guide cell behaviour towards the desired biological response (i.e. increased adhesion and proliferation) [17,18], this feature of the developed material demonstrates its potential in future applications. In fact, the hydrolysis conditions involved in the sol–gel method determine the final state of the condensed particles [19]. The size of the ormoglass particles, for example, can be tailored by changing the quantity of water introduced to initiate hydrolysis [19,20]. Thus, owing to exact control at the hydrolysis stage, it might be possible to modify the topography of the coating. The nanoroughness that can be achieved by coating polymeric fibres with glass particles is essential for the regulation of cellular behaviours [21].

On the other hand, chemical composition of the surface also affects cellular response: the composition of the coating

measured by EDS showed that PLA fibres were coated with 55.1 ± 3.0 Si, 9.5 ± 0.8 P_2 and 35.4 ± 3.9 Ca ormoglass (molar ratio). In this work, rat mesenchymal stem cells (rMSCs) seeded on the material spread generously, demonstrating good interactions with the scaffold even after just 1 day (figure 1*d*–*f*). In addition to the direct exposure of the glass, this copious spreading can be explained by the notable hydrophilic properties achieved after the coating process (figure 2*a*) and the focal adhesions created, thanks to the nanostructured surface of the matrix [22]. In the longer term, the produced material is intended to act as an ion release agent that will promote rMSC differentiation by delivering the appropriate chemical cues [11,12]. In parallel, this material could be used as a drug delivery system to enable the release of therapeutic agents (molecules or particles, for example). The formation of hollow fibres during the electrospinning process (figure 2*b,c*) [15] provides a supplementary surface-to-volume ratio that would enhance the degradation rate of the scaffold, as well as an inner surface that could be functionalized with other bioactive or antimicrobial agents [23].

In addition to cell response, another crucial aspect for the development of functional scaffolds for bone tissue engineering is the mechanical properties. As glass alone is extremely brittle, hybrid materials have been developed to produce glass-containing scaffolds with improved resistance to mechanical failure (toughness) [4]. Having an elastic deformation ability is essential to ensure easy handling and structural integrity of the scaffold during implantation, for example.
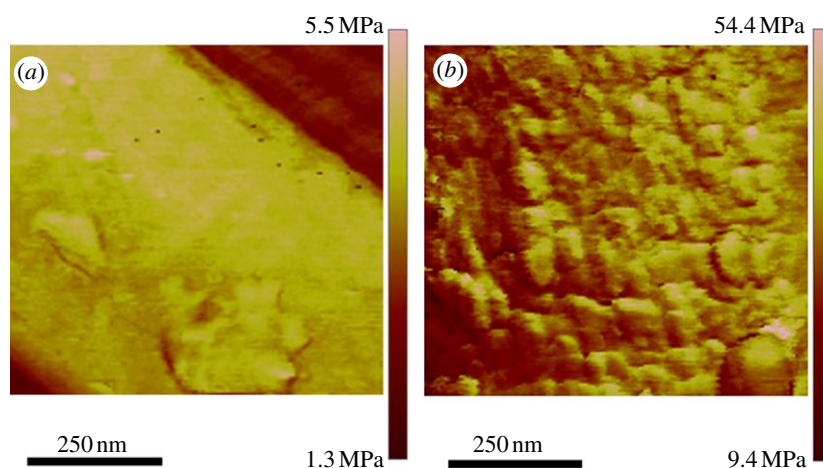
Figure 3. Scaffolds stiffness. AFM images of (*a*) pure PLA fibres (DMT modulus = 3.84 ± 0.32 MPa) and (*b*) coated fibres (DMT modulus = 42.23 ± 4.24 MPa) attesting of the improvement of hardness after coating. (Online version in colour.)

According to mechanical tests (atomic force microscopy), the DMT modulus of PLA scaffold is significantly increased after coating (figure 3*a*,*b*). The material remains however within an interesting working range, where it is ductile and has a low fragility (see electronic supplementary material, table C). It is, in fact, remarkably flexible for a material that has glass as one of its constituents, in comparison with other hybrids (see electronic supplementary material, figure D) [4,24]. This can be explained by the use of an ormoglass rather than a fully inorganic one [25] and the continuity of flexibility maintained between the polymeric structure and its non-brittle coating. No delamination of the glass is observed when bending the material, and it can easily be manipulated to fill bone defects with any shape. The coating approach offers thus the possibility to enhance the stiffness of the pure polymeric structure and reaches values closer to that of the calcified bone while remaining flexible.

3. Conclusions

In summary, the preliminary assays reported herein reveal the great potential of this scaffold to be used as an engineered material for bone tissue engineering. It exhibits a nanostructured topography, excellent hydrophilic properties, remarkable mechanical features and shows, as first encouraging *in vitro* assay, a great ability to support cell spreading. The total exposure of the inorganic compound was achieved, as well as the creation of a covalent bond between the polymer and the glass. This strong interaction is expected to lead to a more homogeneous degradation of the material in body fluid. Moreover, the tubular structure is a significant advantage for the design of multifunctional materials. The novel protocol developed to produce this scaffold is cost effective and can be modified to coat other structures with other glasses. This experimental method constitutes therefore a promising and versatile approach which provides possibilities for a broad range of biomedical applications that require well-defined, hierarchically engineered biomaterials.

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