Bi-functional scaffold for bone regeneration after osteosarcoma resection

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Abstract text (max 5000 characters including spaces)

Introduction
Osteosarcomas (OS) are highly aggressive tumors with a significant prevalence in pediatric patients [1]. OS's therapy usually involves surgery to eradicate tumor mass and subsequently the administration of chemotherapeutic drugs to eliminate potential residual tumor cells. Resection of OS causes large bone defects, therefore there is considerable interest in developing new strategies for the design of bone substitutes able to fill the created void. Moreover, several strategies for the release of drugs have been explored to prevent tumor recurrence. With the aim to address both these needs in a single device, in this work we propose the fabrication of a bicomponent scaffold.
In particular, the device should be able to simultaneously support bone regrowth after OS resection and provide a controlled drug release. To achieve this, a 3D porous structure composed of gellan-gum (GG) embedding magnesium containing-nano hydroxyapatite (Mg-nanoHA) was developed by means of lyophilization technique. The nanoHA was enriched with magnesium ion because of its known role in enhancing the osteogenic response and stimulating osteoblast proliferation, therefore being able to play a key role in bone metabolism [2]. To prevent tumor relapse, the composite sponge has been surrounded by a cylindrical electrospun core-shell membrane able to release a chemotherapeutic drug. Doxorubicin hydrochloride (DOX) was loaded into the polyvinyl alcohol (PVA) core of the fiber while the external shell layer, composed of a poly(ε-caprolactone) (PCL) and chitosan (CS) blend, was design to protect the incorporated DOX and to control its release. *In vitro* tests are currently on going.

Methods
Mg-nanoHA particles were produced by means of a hydrothermal treatment at 100 °C for 4 hours and features a 5% molar magnesium content. An aqueous GG solution (1.5% w/v) was prepared at 90 °C and the Mg-nanoHA particles previously dispersed in distilled water were added to the GG solution creating a Mg-nanoHA_GG suspension that was then crosslinked with a $CaCl_2$ aqueous solution, frozen and lyophilized obtaining a scaffold. The core-shell nanofibers were prepared using a co-axial electrospinning and collected on a rotating mandrel of 5 mm diameter. The shell was composed of a PCL and CS blend in a mixture of acetic and formic acid; the core was prepared adding 0.2% w/v of DOX in a PVA solution composed of water and acetic acid. The electrospinning was performed at a flow rate of 70 µL/h for the core and 210 µL/h for the shell, applying a voltage of 18-20 kV and using a rotating speed of the mandrel of 450 rpm. The electrospun cylindrical matrix was cut with a height of 5 mm. The scaffold was obtained by punching the Mg-nanoHA_GG sponge in a cylindrical shape of 5 mm diameter and 5 mm heigh, that was then inserted into the cylindrical electrospun matrix.

Results
The synthetized Mg-nanoHA particles had uniform size of 70-90 nm in length and are 20 nm wide, and the incorporated magnesium was very close to the theorical content. XRD confirmed the presence of the typical peaks of HA and excluded the formation of any secondary phases during the synthesis. The Mg-nanoHA_GG composite sponge was highly porous and micro-CT revealed a homogeneous and interconnected porosity with pores of dimension around 200-300 µm. The composite sponge showed high water absorption (over 1000%) and limited swelling abilities (around 5%). The electrospun fibers had uniform dimension around 250-400 nm and TEM was able to assess the core-shell structure. A sustained DOX release up to 28 days was observed with a limited burst release. The complete scaffold did not show collapse after hydration and the swelling of the composite sponge provided a satisfactory cohesion with the outer electrospun layer.

Conclusions
A bi-component scaffold using two different processing techniques was obtained. Lyophilization allows the production of a porous sponge that serves as a bone filler, providing a porous 3D structure and supporting the osteogenic response due to the presence of Mg-nanoHA. The external core-shell electrospun layer was fabricated with PVA and a PCL-CS

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blend, widely used as biomaterials due to their known properties of biodegradability and biocompatibility and acting as a local release depot of DOX. The combination of these two processing techniques allowed the development of a multifunctional 3D scaffold that can possibly support the growth of new bone tissue and the sustained release of therapeutic agents to prevent tumor relapse.

References:
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