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How digital twins can optimize stimuli to accelerate bone regeneration?

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Abstract - **Digital twins (DT) that mimic biophysical cellular environments is an exciting new approach in regenerative medicine, in particular for bone tissue engineering. The possibility of exploring different designs of scaffolds and bioreactors, is of paramount importance in this area, while simultaneously also understanding the effects of applying external stimuli, such as mechanical or electromagnetic. The use of DT enables critical design optimization before reaching the laboratory bench, thus saving time, costs and life resources. We present an overview of the application of DT in bone regeneration. The relevant biocompatible materials, most commonly used design of scaffolds and bioreactors and the latest results regarding stimuli application are discussed.**

I. Scaffolds role in tissue engineering

Tissue engineering (TE) uses living cells seeded on 3D scaffolds to facilitate cell adhesion, growth, differentiation, proliferation and/or alignment. Scaffolds can be placed on an optimized bioreactor, a closed system that mimics cellular biological environment and through which different types of external stimuli (such as mechanical, electrical and magnetic) can be applied to accelerate cellular processes [1]. Scaffold design can be of paramount importance to guarantee the success of tissue regeneration. Properties such as biocompatibility, degradation time and biomechanical properties of target tissues should be carefully considered when designing and fabricating scaffolds. For instance, in bone TE, porosity and mechanical stiffness have to be adequately balanced to facilitate cell adhesion and differentiation, thus reproducing realistic bone physical phenotype. Topological optimization calculations can fine-tune scaffold geometry aiming at the best predicted outcome [2].

The production of scaffolds have recently evolved with 3D printing techniques, allowing highly complex and optimized manufacture of nano-structures possible- for instance, using technologies such as Additive Manufacturing (AM). Depending on the materials used (polymer, metal, ceramic or composite), scaffold fabrication techniques differ immensely. For polymers and their composites, 3D printing techniques can be employed for scaffold fabrication. Further, polymer scaffolds with a biomimetic coating can also be used for bone TE. Additionally, electrospinning has been shown to be versatile for making nanofibrous scaffolds that allow cells to be incorporated in electrospun scaffolds. This enables the manufacture of multilayered scaffolds in neural TE and of graded scaffolds for osteochondral TE.

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II. Bioreactors-based tissue engineering

The design, materials and process chosen to manufacture bioreactors play also a key role in tissue regeneration. A new generation of bioreactors are currently being produced, aiming at the application of mechanical, electrical and magnetic stimuli, either isolated or combined [3] (Fig.1). Digital twins provide a theoretical framework to guide the design of the bioreactor using aforementioned different components. This approach results in providing adequate stimuli delivery at a region-of-interest (ROI) – exactly where the scaffold with cells would be located. In the case of combined mechanical-electrical stimulation, geometry of electrodes and fluid flow perfusion system can be optimized to ensure adequate electric field and fluid shear stress values are delivered- i.e. within the scaffold known to accelerate cellular adhesion, proliferation, growth and differentiation [3].

The discussion on different types of scaffold and bioreactor designs is essential to identify future trends in the development of a combined modelling-experimental approach. Therefore, the creation of DT holds great promise for solving many difficulties concerning regenerative medicine applications.

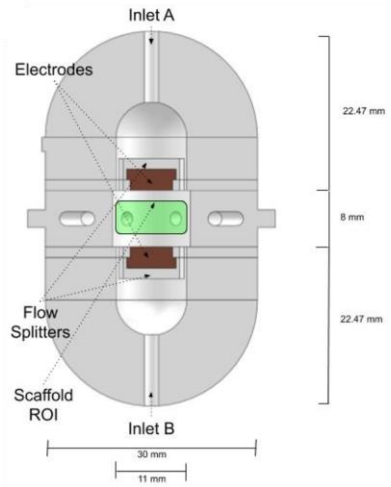

Fig.1 Bioreactor design: vertical cut view, illustrating parallel electrodes set up, upper and bottom inlets and inlet flow splitters [3].

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Muscle coordination and loading patterns after bone defects in sheep model via a biomechanical musculoskeletal approach

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***Abstract* - Computer-generated simulations are used to predict the various possible biological and toxic effects and in a final stage, animal testing is done to obtain confirmatory results. The study of mechanical loads and their influence on the bone meshwork is important to increase the understanding of the mechanical consequences, bone remodeling and loss after orthopedic implantation. This work aims to present a musculoskeletal model of both sheep's hindlimbs to understand loading mechanisms and muscle coordination patterns in healthy sheep gait.**

I. INTRODUCTION

Musculoskeletal modeling of the sheeps' hindlimbs with the intent of estimating tibiofemoral contact forces whilst walking at different speeds on a treadmill is scarcely documented [1]. This study inferred that tibiofemoral contact forces increase with walking speed at different rates, with vertical contact forces increasing at a faster pace. On the other hand, by aiming to standardize limb loading in sheep using an instrumented treadmill, they make it uncomfortable for the sheep to walk naturally or even comply to the task in hand

II. METHODS

The musculoskeletal model hereby presented describes both hindlimbs of a sheep, combining fifteen rigid bodies (pelvis; proximal phalanges; metatarsus; talus; tibia; patella; femur; centroquartal bone, and first, second, and third tarsal bones), connected by joints, resulting in twelve degrees of freedom overall. Sixty-two musculotendon actuators are employed to represent the hindlimbs muscles and drive the model through the simulations. Muscle parameters, such as maximum isometric force and pennation angle, are adapted from previous literature [1].

III. RESULTS

Kinematic and force plate data were acquired using a set of healthy sheep, walking at their own natural pace. Kinematic data was recorded using the coordinates of reflective markers recorded with the optoelectronic stereophotogrammetry motion capture system. Muscle and contact forces were estimated using OpenSim [2]. The musculoskeletal model was scaled to match each sheep's anthropometry. A residual reduction algorithm (RRA) step was used to minimize errors related to kinematic inconsistencies and modelling assumptions. Bone – on – bone forces will also be calculated using OpenSim. An induced acceleration analysis (IAA) was performed to estimate muscle contributions to the acceleration of the centre of mass. A rolling constraint without slipping will be inserted in this analysis to substitute the interaction of the musculoskeletal model with the surrounding environment. Muscle forces were estimated using Computed Muscle Control (CMC) optimization technique for this analysis [3].

IV. DISCUSSION & CONCLUSION

By using the resultant joint contact forces from the biomechanical analysis using the musculoskeletal model in a finite element analysis, one is able to integrate a finite element model that is stimulus – responsive to mechanical loads. In addition to this, this framework is also helpful in designing, optimizing and validating scaffolds for bone regeneration, which help reduce the complications inherent to the occurrence of bone defects. Also, being able to replicate in vivo occurrences of mechanical loading via computational models is key to reduce the number of animals necessary for this study and optimize therapeutic solutions.

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Tissue Engineering Materials and Scaffolds and their Developments

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Abstract - **This paper gives an overview of the development of tissue engineering scaffolds that are produced using a variety of technologies. It focuses on employing 3D printing to fabricate advanced scaffolds for regenerating complex body tissues.**

I. SCAFFOLD-BASED TISSUE ENGINEERING

Tissue engineering (TE) uses living cells and extracellular components to form implantable devices for human body tissue regeneration. It holds great promises for solving many difficult medical problems. Different TE strategies, including factor-based, scaffold-based, cell-based or their combination, are investigated (Fig.1a). Scaffold-based TE employs porous scaffolds to promote cell ingrowth, extracellular matrix deposition and tissue formation. There is a set of criteria for TE scaffolds, including a highly porous structure having a 3D interconnecting pore network of suitable pore sizes. Scaffolds are mainly made from biomedical polymers, with natural polymers being favored (Fig.1b). New biomaterials, such as composites and hybrids, are made and studied for tissue engineering applications [4]. For example, osteoconductive composites are developed for making scaffolds for bone regeneration (Fig.1c).

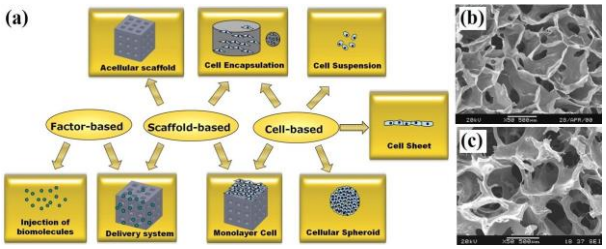

Fig.1 Tissue engineering: (a) TE strategies [1], (b) scaffold made from chitin [2], (c) scaffold made from psHA/PDLLA composite [3].

II. SCAFFOLD FABRICATION TECHNOLOGIES

Depending on the material or materials (polymer, metal, ceramic or composite) to be used for a TE scaffold, scaffold fabrication techniques differ greatly. For polymers and their composites, techniques such as freeze-drying [5], electrospinning [6] and 3D printing [7] can be employed for scaffold fabrication. Polymer scaffolds with a biomimetic coating can also be formed for bone TE [8]. Electrospinning is versatile for making nanofibrous scaffolds and cells can be incorporated in electrospun scaffolds [9]. It enables formation of multilayered scaffolds for neural TE [10].

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III. 3D PRINTING IN TISSUE ENGINEERING

3D printing is a power platform for tissue engineering. Scaffolds can be made using different 3D printing technologies, and bioactive molecules such as growth factors (GFs) can be encapsulated (Fig.2). 3D printing also enables construction of graded scaffolds for osteochondral TE [12].

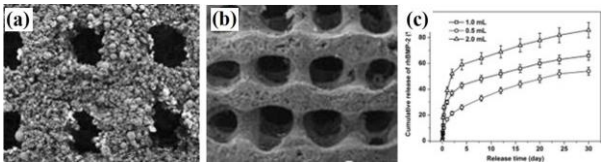

Fig.2 3D printed scaffolds: (a) scaffold formed by SLS [7], (b) scaffold formed by extrusion [11], (c) growth factor release from scaffolds [11].

4D printing produces shape-morphing scaffolds to meet the demanding requirements in particularly TE applications [13]. Self-folding tubular scaffolds with controlled GF release can be printed for vascular TE (Fig.3).

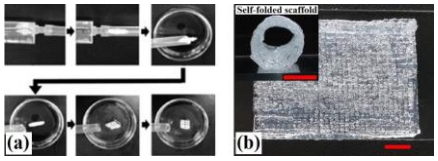

Fig.3 4D printing of TE scaffolds: (a) shape recovery of a 4D printed scaffold [14], (b) self-folded tubular scaffold [15].

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The use of Magnetic Stimulation for in vitro bone tissue engineering

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***Abstract*— The influence of magnetic fields on biological behavior of bone tissue has been a topic of considerable interest for many years. In order to investigate the range of parameters considered across various cell types and its resultant effects, a systematic review of articles was performed. While we observed that most studies were efficacious, a wide variety of parameters have been employed ranging from magnetic field strength, frequency, duration, and days of stimulation.**

I. INTRODUCTION

In context of tissue engineering, the proliferation and/or differentiation of bone-related cells is modulated by several factors, such as scaffold design, growth factor, culture system, physical stimulation modalities, etc. Of the various stimulation approaches, magnetic fields offer promising option for bone regeneration. Multiple studies indicate that magnetic fields stimulate proliferation and differentiation of osteoblasts, promote growth factor expression, increase osteointegration and accelerate new bone formation [1-3]. The main aim of this work was to review the current state-of-art of magnetic stimulation approaches in bone tissue engineering restricted to in vitro approaches. Specifically, we were interested in quantifying the range of stimulation parameters and effects observed. This information would ultimately help plan for range of tissue engineering experiments involving multiple stimulation modalities (mechanical, electrical, magnetic) as part of a bigger project.

II. METHODS

The systematic review involved an eligibility criteria including availability of full articles, article in English, and included all studies listed on electronic databases as of the date of the search. The exclusion criteria consisted of review articles, in vivo studies, study not related to bone regeneration, usage of stimulation other than magnetic stimulation. The search was carried out on July 12' 2020 using the following keyword search "osteogenesis OR osteogenic AND magnetic stimulation AND in vitro". Researcher (A.D.) conducted the search in the PubMED electronic database, which is the most extensive source for search and retrieval of literature relevant to the subject topic.

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III. RESULTS

The initial search using the aforementioned keyword combination resulted in 59 results. Out of these, 35 were excluded in accordance with the adopted inclusion and exclusion criteria. Of the 14 that were analyzed, 4 studies used static magnetic field (SMF), 7 used pulsed electromagnetic filed (PEMF), 2 used a combination of SMF and magnetic nanoparticles (MNP), and 1 used alternating magnetic field. While most PEMF studies employed a commercially available pulse generator, most SMF studies used neodymium magnets to generate the magnetic field. A bioreactor was used for the alternating magnetic field study. The most common cell type considered was osteoblast (8 studies), with mesenchymal stem cells (MSC) used in 3 studies. The remaining studies used adipose derived stem cells – either independently or in combination with MSC. The magnetic field strength for PEMF studies varied between 0.2 mT – 1 T, with the most common value being 2 mT. The magnetic field strength for SMF and SMF in combination with MNP studies ranged between 15 mT – 280 mT with the exception of the one study that used a superconducting magnet that generated strengths as high as 8T. We observed no one common value across studies with most testing a range. The magnetic field strength induced in the alternating magnetic field study was ~25 mT. In terms of stimulation duration, the range varied between 1.5 hours to 24 hours per day over multiple days (maximum 30 days). The frequency of PEMF stimulation (not relevant for SMF stimulation) varied between 50 – 75 Hz with 75 Hz being the most common value. Overall studies indicate that magnetic stimulation enhance bone regeneration from cell proliferation, differentiation, extracellular matrix production, mineralization, gene expression, etc. Stimulation parameters can result in non-linear effects and need to be carefully considered.

IV. DISCUSSION & CONCLUSION

Based on the data analysis, it is possible to conclude that overall magnetic stimulation has been shown to be efficacious for bone tissue engineering ranging from osteoblast proliferation to promoting differentiation and mineralization. The ideal stimulation parameters to use continues to be an active research topic.

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Coaxial Poly(glycerol sebacate) /Polycaprolactone-Polyaniline fibers for neural applications with induced pluripotent stem cells

Fábio F. F. Garrudo, Diogo E. S. Nogueira, Carlos A. V. Rodrigues, Flávio A. Ferreira, Mário Vale, Ana C. Marques, Joaquim M. S. Cabral, Jorge Morgado, Robert J. Linhardt, *Frederico C. Ferreira***

***Abstract*— We report the development of electrospun biodegradable and electroconductive coaxial nanofibers composed of an external layer of polycaprolactone (PCL) and polyaniline (PANI) and a core layer composed of poly(glycerol sebacate) (PGS). Neural differentiation of induced pluripotent stem cells (iPSCs) under electrical stimulation was promoted, and the cells acquired a glutamatergic phenotype instead of a typical GABAergic/dopaminergic one.**

***Clinical Relevance*— Electrical stimulation can be used to direct neural cell differentiation profile. Electrical stimulation can potentially enhance the safety of iPSC-based therapies.**

I. INTRODUCTION

Chronic diseases, such as neurological diseases, have a prominent impact on the general population. In particular, neurodegenerative diseases remain incompletely understood and ineffectively treated. Replenishing neurons in the brain may represent the best therapy for these progressive and fatal diseases. Clinical trials show that implanted cell integration into patient's brain tissue is limited by poor cell survival. This challenge can be addressed by the development of new scaffolds, with enhanced biomimetic properties using mechanical/electrical cues to direct stem cell differentiation into neurons.

II. METHODS

The present study aimed at developing biocompatible PANI based coaxial electrospun fibers for neuron regeneration. The work was developed in three stages: (1) optimization of the best PCL to PANI ratio for an optimal electroconductivity [1]; (2) optimization of the solvent system to enhance electroconductivity [2]; and (3) production of co-axial fibers, composed by an external conductive layer of PCL-PANI and an internal layer of PGS [3,4]. The fibers' physico-chemical properties were then investigated using scanning electron microscopy (SEM), Fourier transform infrared analysis (FTIR) and differential scanning calorimetry (DSC), followed by electrical and mechanical characterization. *In vitro* stability and biodegradation were also assessed. The obtained fibers were then used for the culture of induced neural progenitors (differentiated from F002.1A.13 iPSCs, p42) for 30 days under electrical stimulation (pulsed DC, 1 V cm^{-1}, 100 Hz).

III. RESULTS

Coaxial PCL-PANI/PGS fibers were produced. Average diameter was 951 ± 465 nm and electroconductivity 0.063 ± 0.029 S cm^{-1}. The mechanical properties (ε = 1.3 MPa) and hydrophilicity (38 º) favor neural cell culture. The neural differentiation of iPSCs was favored on PGS/PCL-PANI fibers. Electrical stimulation induced an up-regulation of glutamatergic markers (15-fold) and voltage-sensitive channels (12 for SCN1A, 32-fold CACNA1C) and down regulation of GABAergic marker (11 fold).

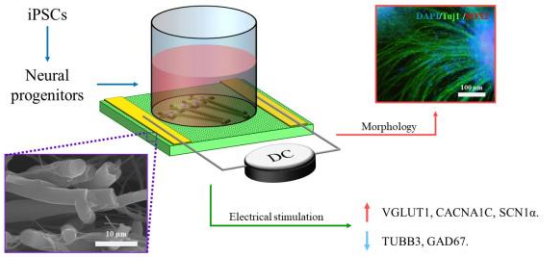

Figure 1. Schematics of the work developed.

IV. DISCUSSION & CONCLUSION

Our results show that the fibers developed have potential applications in neural tissue engineering: (1) to build reliable *in-vitro* platforms for drug screening; (2) interfaces for deep-brain electrodes; and (3) for direct transplantation of mature and functional neurons into patients' brains.

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