Advances in Bone Tissue Engineering with Renewable PlasCarbon

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PlasCarb is an EU-funded project which aims at the development of a technology that transforms biodegradable food waste into high-purity graphitic carbon through microwave plasma splitting of biogas. The final product, Renewable PlasCarbon (RPC), has since been demonstrated in numerous researches to be an outstanding basis for graphitic substances (eg. inks, coatings and battery cathodes). The present test report by Abalonyx showcases that RPC can be also applied in the medical field: as a reinforcement to bioactive glass, used mainly for bone tissue engineering, Renewable PlasCarbon seems to provide superior properties to conventional ceramic scaffolds.

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Introduction

The underlying idea of this work is to fabricate three-dimensional porous structures called 'scaffold' which provide the mechanical support during repair and regeneration of damaged or diseased bone. Abalonyx has prepared these 3D scaffolds based on 45S5 Bioglass® reinforced with Renewable PlasCarbon for bone tissue engineering.

Bone is one of the most frequently transplanted tissues, and the demand is steeply rising due to the rapidly upward trend of worldwide incidence of bone lesions especially in populations where aging is coupled with increased obesity and poor physical activity. Bioinert bone implants like metals (e.g. Ti), or oxide ceramics (e.g. Al_2O_3, TiO_2**)** and bone grafts are utilized in a wide array of clinical settings to enhance bone repair and regeneration. However, they have their own limitations such as limited materials, a need for a second operation or immunogenic reactions and disease transmission risks [1][2]. New methods are thus needed to overcome these problems and meet the growing demand.

Bone tissue engineering in the medical field

The field of bone tissue engineering (BTE) has emerged nearly three decades ago as a convenient alternative to promote the regenerative ability of the host body. One of the most important stages of BTE is the design and processing of a porous, biodegradable three-dimensional (3D) structure called 'scaffold', exhibiting high pore interconnectivity and uniform pore distribution, which provides the mechanical support during repair and regeneration of damaged or diseased bone.

Within the regenerative medicine market, the worldwide value attributed to products in the orthopedic market was over €2 billion in 2006. Three years later, the market value limited to biomaterials used in orthopedic applications has been valued approximately $3.5 billion in the United States of America alone. Globally, this market is projected to grow to $9.6 billion by 2016 at 12 % CAGR. A skeletal tissue regeneration product based on 3D scaffolds can be placed in the same commercial segment, which takes up about 85 % of the market share with further growth to be expected [3]. As a simplistic example, assuming 10 million operations per year worldwide, already in the near future - with each scaffold being worth $1000 - this would represent a value of $10 billion.

Conventional scaffolds and their reinforcement with graphene

Currently, biodegradable bone scaffolds are processed either from ceramics (calcium phosphates or bioactive glasses), polymers or their composites [2][4][5]. Ceramic scaffolds show a greater potential for bone tissue engineering applications because of their excellent ability to bond directly to bone tissue and their higher elastic moduli [6][7]. In conventional techniques for manufacturing these bio-ceramic scaffolds (such as foam replication), having a precise control over the pore structure like pore size, geometry, and spatial distribution is not achievable, which leads to producing scaffolds with very high porosities and consequently very low mechanical properties [8].

Fortunately, rapid prototyping (RP) technologies which have been recently developed can overcome these hurdles [9][10] since they build structures layer-by-layer with customized and complex 3D shapes following a computer-aided design (CAD) model. They can therefore produce the optimal pore architecture to attain the desired mechanical and diffusion properties for a given application. Moreover the CAD model can be obtained from medical scan data (computerized tomography, nuclear magnetic resonance imaging, etc.), allowing the scaffold's external shape to match the damaged tissue site. Among the RP techniques capable of building ceramic scaffolds, robocasting (also known as direct-write assembly) is unique because it allows one to build scaffolds using inks (highly concentrated suspension of desired materials for the fabrication of the scaffolds) with minimal organic content (< 1 wt.%) and capable of fully supporting their own weight during assembly [11][12].

Nonetheless, despite the improvement in pore architecture and consequently mechanical properties achieved by robocasting, the main limitation of these ceramic scaffolds still lies in their intrinsic brittleness and, thus, low resistance to crack propagation, which makes most of these scaffolds unsuitable for load-bearing applications [13]. Reinforcing the bioceramic scaffolds by graphene is one solution for tackling this problem. Graphene as the emerging carbon nanomaterial is a promising candidate which possesses excellent mechanical properties far superior to other known reinforcements in:

- transferring their mechanical properties to the host material;
- covering a large surface area;
- electrical property (conductivity);
- the ease for chemical modifications [14].

Recent findings demonstrated that graphene can enhance bioactivity and differentiative potential for bone tissue regeneration, and has no adverse effect *in vitro* and *in vivo* [15]. It has been also shown that graphene has exceptional properties as medium for human neural stem cells as it has enhanced their formation rate significantly [16]. Moreover, these electrically conductive nano-composites can facilitate cell growth and bone tissue regeneration with the help of physioelectrical signal transfer: when bone is subjected to mechanical stresses, its deformation leads to electric signals (piezoelectric effect), which can therefore contribute to bone regeneration and fracture healing [17]. Therefore, composites of bioceramics/graphene could be an alternative for existing composites for bone tissue engineering applications. Consequently, the aim of this research work is to develop novel hybrid biomaterials by robocasting technique based on 45S5 Bioglass® (a silica glass with the composition of 45wt.% SiO_2, 24.5wt% CaO, 24.5wt% Na_2O and 6wt% P_2O_5) reinforced with Renewable PlasCarbon, which structurally is very similar to graphene as discussed in Hof *et al.* (2016) [18].

Testing procedure

As shown in Figure 1, a robocasting device was employed to fabricate 3D structures by layer-wise deposition of the optimized 45S5 bioglass/graphene ink. The ink was housed in a syringe and extruded through a conical nozzle by the computer-controlled robotic system. The external dimensions of the scaffolds were set at about 20 × 20 × 10 mm, and the design consisted of a tetragonal mesh of cylindrical rods with a center-to-center spacing between adjacent rods within a layer of 820 µm and a layer height of 287 µm.

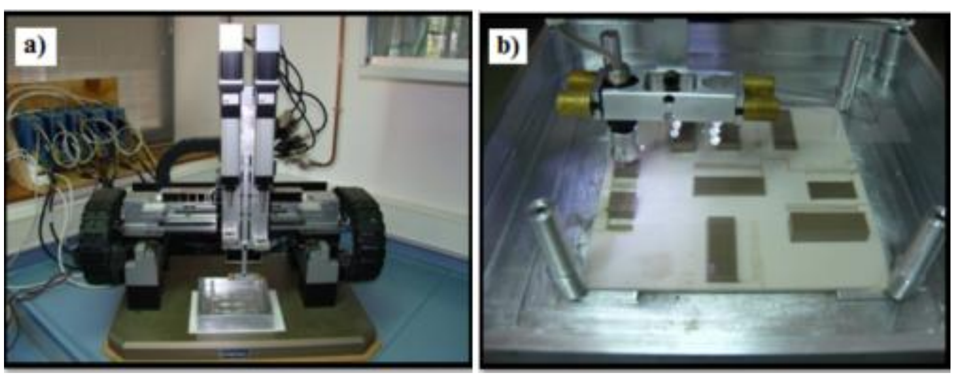

Figure 1. Optical images of (a) the robocasting system, (b) 3D robocast structures as-deposited within an oil bath.

To strengthen the structures, scaffolds were pressureless sintered at 1000 °C in a resistance-heated furnace under a flowing argon atmosphere. Figure 2 shows three dimensional porous scaffolds consisting of multiple layers that were produced by robocasting using (a) 45S5 bioglass and (b) 45S5 bioglass/graphene inks.

a b

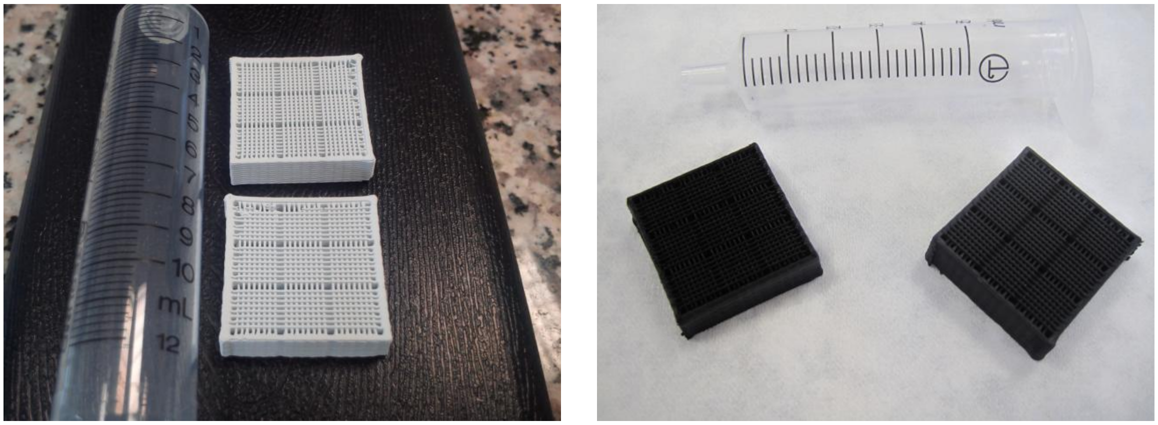

Figure 2. Optical image of 3D porous scaffolds produced by robocasting from (a) 45S5 bioglass ink and (b) 45S5 bioglass/graphene ink.

Conclusion

This study showed that the addition of 1 vol% Renewable PlasCarbon increased the compressive strength and fracture toughness of hybrid scaffolds by 45 % and 280 % respectively compared to pure bioglass scaffolds. It was also shown that the hybrid scaffolds were electrically conductive with resistivity of ~ 0.2 Ωm.

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