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Short Communication

Tailoring Scaffold Porosity for Tissue Regeneration

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Abstract

Scaffold porosity is an essential factor for tissue growth since it allows cell proliferation and vascularization. In this work, three different scaffolds were produced by easy methods. The first one was prepared using Bioglass 45S5 particles and H2O2 as a foaming agent. In the second, glass nanoparticles were arranged as porous structure by the self-assembling of cellulose nanocrystals. In the last, a polymeric xanthan/chitosan composite scaffold was prepared by swelling method. The results showed that the scaffolds presented pore morphology, wall thickness and size distribution that are dependent on the materials and preparation method used, and that they are suitable for a range of applications.

ABBREVIATIONS

CNC: Cellulose Nanocrystals; CTAB: Hexil Dodeciltrimetilammonium Bromide; TEP: Triethyl Phosphate; TEOS: Tetraethyl Orthosilicate

INTRODUCTION

Several studies have shown the importance of scaffolds to repair bone and other tissue damages [1,2]. They are commonly used to support tissue cells, allowing cellular proliferation as well as the diffusion of nutrients and waste [3,4]. One of the most important characteristics of scaffolds is their porosity, which is essential for vascularization during new tissue growth [5,6]. Suitable pore size distributions and shapes can be reached by using different methods to obtain scaffolds [1-6]. such as electrospinning [7]. foam replication [8]. and 3D printing [9,10]. Furthermore, there are some methods that can easily produce scaffolds with controlled pore size and morphology by using simple approaches which change some of the conditions during the scaffold formation, tailoring them for the appropriate application [6-11]. In this communication, three scaffold preparation methods are presented. These methods can be applied in order to tailor the pore size, wall thickness and morphology. The methods use a porogenic agent, cellulose nanocrystals as a template and also polymer swelling followed by freeze-drying process. These methodologies can be used to prepare scaffolds from inorganic and/or organic materials such as bioglass and biopolymers. Namely, using Bioglass® 45S5 particles and a porogenic agent, it is possible to obtain scaffolds with pores ranging from tens to hundreds of micrometers. Alternatively, Stöber silica nanoparticles [12]. can be used to prepare glass nanoparticles that coat and copy the self organized CNC arrangement, forming scaffolds with pore size of tens of micrometers. Meanwhile, xanthan-chitosan composite swelling and freeze-drying can have their pore size controlled by changing the pH of the swelling solutions [5-11]. In this work, different scaffolds were produced using these above mentioned methods and these can be used to heal different tissue damages.

MATERIALS AND METHODS

Scaffold production using Bioglass® 45S5 particles

The scaffold was produced using 45S5 particles, which were produced by melt-quenching as vastly described in literature [13]. using SiO_2, $CaCO_3$, Na_2CO_3 and P_2O_5 (Sigma-Aldrich). The melted glass was immediately poured into deionized water (Fritas method), washed with ethanol and dried. These particles were milled in a high energy ball mill and sieved in order to obtain 53 to 106 μm particle diameters. The scaffold was built up by placing the particles in a mold with a 1% (v/v) H_2O_2 (Synth), 1% (w/w) Ultranex 120 (Oxiteno) and 2M NaOH (Synth) solution in a large enough quantity to cover all the particles. The base volume excess was removed after 24 h and the system dried for another 24 h. Finally, the product was calcined at 1000ºC for 2h.

Synthesis of glass scaffolds using glass nanoparticles and cellulose nanocrystals as template

Glass scaffolds were produced by combining surface modification of cellulose nanocrystals with the cationic surfactant CTAB and glass nanoparticle synthesis by the Stöber method [12]. The experimental procedure was carried out by dispersing 1.30 g CNC aqueous gel (University of Maine-USA) in a mixture of 20 mL deionized water and 80 mL ethanol (98%). Then, 0.49 g CTAB (Sigma-Aldrich) was added and the suspension was stirred for

10 minutes. After that, 10mL 4.2% (v/v) NH_4OH and 100µL 1% TEP (Sigma- Aldrich) aqueous solutions and 70µL TEOS (Sigma-Aldrich) were added to the suspension and stirred for 20h. The suspension was centrifuged at 1000 rpm and 100µL 1% calcium nitrate solution (Sigma-Aldrich) was added and the suspension was stirred overnight. The suspension was again centrifuged and the precipitate was freeze-dried and calcined at 600ºC for 6h to remove the organic compounds and to produce the glass scaffolds.

Assembling porous scaffolds of xanthan and chitosan produced by layer-by-layer and swelling/freezing drying

Films made up of xanthan gum and chitosan layers were prepared using dip-coating and layer-by-layer methods. Xanthan hydrogel was obtained by a preparation of 1% (w/v) xanthan gum (Mapric, Brazil) aqueous solution and chitosan hydrogel was prepared from a 2 % (w/v) chitosan (Sigma Aldrich, Brazil) aqueous acetic acid (Synth, Brazil) solution. To make the film, a glass slide was used as a substrate and dipped in the xanthan hydrogel, dried in a chamber with nitrogen flow, and then dipped into chitosan hydrogel followed by another drying step. This process was repeated twice to obtain three xanthan/chitosan composite layers. The final film was swelled in deionized water for 48 hours and freeze-dried in order to produce porous scaffolds.

RESULTS AND DISCUSSION

SEM images of the scaffolds made up of 45S5 particles (Figure 1A and 1B), glass scaffolds prepared using CNC as a template (Figure 1C and 1D), and the scaffolds prepared with swelled xanthan-chitosan film (Figure 1E and 1F) are shown in Figure 1. Scaffolds with different pore morphologies are formed, depending on the precursor type and methodology. Figure 1A and 1B show Bioglass® connected particles, indicating that a reaction between the NaOH and the glass particles promoted the softening of their surfaces, resulting in densely connected microparticles. These connected particles led to the formation of a scaffold that presents good mechanical strength and large pores. The use of Bioglass® 45S5 particles, one of the best known materials for bone repair, combined with surfactant and base, can be considered a very simple method that allows overcoming the current issue of a 45S5 scaffold production. By changing the amount of hydrogen peroxide added, which promoted gas release for pore formation, it is possible to tailor the pore sizes. The scaffold produced from glass nanoparticles, which copied the cellulose nanocrystal arrangement template, are presented in Figure 1C and 1D. This structure, with pores in the order of tens micrometers, was formed by self-assembling after nanocrystal surface modification with CTAB. This method has the advantage of using self-assembly cellulose nanocrystals which can be removed by calcination. Furthermore, cellulose nanocrystals can be easily functionalized and combined with different glass precursors [14,15]. SEM micrographs of the xanthan-chitosan scaffold are shown in Figure 1E and 1F. This material presents fibrous morphology with pores sizes ranging from 0.4 to 6.0µm, which were formed by water swelling followed by freeze-drying. The swelling effect is due to polysaccharide charged groups which are hydrated by diffusing water molecules into the material [5,11,16]. The swelling method has the advantage of controlling the pore distribution by changing some conditions, such as: polymer concentrations of the xanthan and chitosan hydrogels, swelling medium pH and swelling time [11]. The pore wall thickness is another important factor to be considered. The thicker the wall, the more material that has to be phagocytosed by the cells if the scaffold is biodegradable. Additionally, low free volume for cells diffusion will require a higher scaffold biodegradation time. On the other hand, when the walls are thinner, the available area for cell proliferation is higher, which facilitates cell diffusion and growth and,

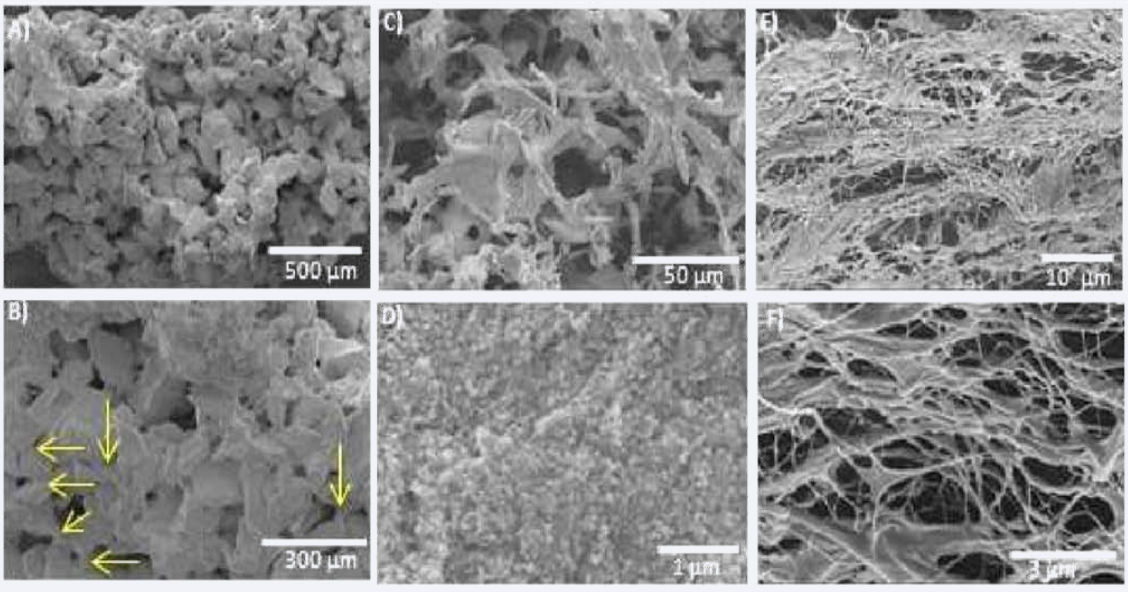

Figure 1 Scanning electron micrographs of scaffolds produced by: Bioglass® 45S5 particles, with the arrows showing the formed necks (A,B), glass from CNC template (C,D) and xanthan/chitosan swelled film (E,F).

consequently, requires less time for scaffold biodegradation [17]. The three methods used in this work produced scaffolds with different pore wall thickness, which makes each one appropriate for different applications depending on the necessary absorption time. The Bioglass® 45S5 scaffolds showed a densely packed porous structure with thick walls, whereas the xanthan/chitosan composites presented a thin walled layered porous structure.

CONCLUSION

In this work, scaffolds with distinct porous morphologies were obtained. The three methods presented are facile, present great versatility and can be used to produce a porous material that can be useful as scaffolds for a number of applications, since the pore size can vary from nanometers to hundreds of micrometers. Additionally, the pore wall thickness could also be tailored to control the time of scaffold biodegradation and cell diffusion.

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REFERENCES

1. Bacaglia LG, Smith BT, Watson E, Arumugasaamy N, Mikos AG, Fisher JP. 3D printing for the design and fabrication of polymer-based gradient scaffolds. Acta Biomater. 2017; 56: 3-13.
2. Gerhardt LC, Boccaccini AR. Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering. Materials (Basel). 2010; 3: 3867-3910.
3. Loh QL, Choong C. Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Eng Part B Rev. 2013; 19: 485-502.
4. Zhang W, Wray LS, Rnjak-Kovacina J, Xu L, Zou D, Wang S, et al. Vascularization of hollow channel-modified porous silk scaffolds with endothelial cells for tissue regeneration. Biomaterials. 2015; 56: 68-77.
5. Turkmen AK, Cavalu S, Goller G. Development of chitosan-hydroxyapatite-fibrinogen 3D scaffolds for bone tissue regeneration. Journal of The Australian Ceramic Society. 2016; 52: 34-41.
6. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005; 26: 5474-5491.
7. Lannutti J, Reneker D, Ma T, Tomasko D, Farson D. Eletrospinning for tissue engineering scaffolds. Materials Science and Engineering C. 2007; 27: 504-509.
8. Lam CXF, Mo XM, Teoh SH, Hutmacher DW. Scaffold development using 3D printing with a starch-based polymer. Materials Science and Engineering C. 2002; 20: 49-56.
9. Sulong MA, Belova LV, Boccaccini AR, Murch GE, Fiedler T. A model of the mechanical degradation of foam replicated scaffolds. Journal of Materials Science. 2016; 51: 3824-3835.
10. Roohani-Esfahani S-I, Newman P, Zreiqat H. Design and fabrication of 3D printed scaffolds with a mechanical strength comparable to cortical bone to repair large bone defects. Scientific Reports. 2016; 6: 1-8.
11. Mendonça RH, Meiga TO, Costa MF, Thiré RMSM. Production of 3D scaffolds applied to tissue engineering using chitosan swelling as a porogenic agent. Journal of Applied Polymer Science. 2013; 129: 614-625.
12. Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. Journal of Colloid and Interface Science. 1968; 26: 62-69.
13. Bourhis, L. Glass Mechanics and Technology. Wiley-VCH Verlag GmbH & Co. 2008.
14. Fu G, He A, Jin Y, Cheng Q, Song J. Fabrication of Hollow silica nanorods using nanocrystalline cellulose as templates. Bioresources. 2012; 7: 2319-2329.
15. Dufresne A. Nanocellulose: A new ageless bionanomaterial. Materials Today. 2013; 16: 220-227.
16. Berger J, Reist M, Mayer JM, Felt O, Gurny R. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur J Pharm Biopharm. 2004; 57: 35-52.
17. Planell JA. Bone repair biomaterials. CRC Press. 2009.

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