STRUCTURAL VARIABILITY OF LYOPHILIZED COLLAGEN-BASED SCAFFOLDS: MICRO-CT 3D ANALYSIS

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Introduction

Bone tissue engineering aims at regeneration of bone defects by application of biomaterials [1]. Structural parameters (e.g. porosity) are expected to have significant influence on scaffold-tissue interaction [2]. Lyophilisation is frequent fabrication method of tissue engineering scaffolds [3,4]. Micro-CT is an emerging non-destructive imaging method applicable for scaffolds structure evaluation [5]. The resulting 3D structure depends on lyophilisation parameters (e.g. temperature). Variability of structure in scaffolds prepared via the same procedure is not well described to date. Aim of this project is to assess the most convenient fabrication method for preparation of homogeneous 3D structure and evaluate its variability, which may limit its application in bone surgery.

Materials and Methods

Collagen-based scaffolds were prepared by means of lyophilisation using 3 different temperatures (-30°C, -80°C, -190°C; in each group were 20 specimens of cylindrical shape, h=7mm, d=6mm). Each specimen was micro-CT scanned (Bruker micro-CT SkyScan 1272, Belgium, Kontich) in air mounted on specimen holder-with following parameters: 4 um pixel size, frame averaging 5, no filter, scanning time approx. 1 hour. Image data were reconstructed and structural parameters (Structure separation, Structure thickness, Porosity, Object surface density) were evaluated by means of 3D analysis using Bruker software. Pore size was analysed based on sphere-fitting algorithm. Results were statistically evaluated (Kruskal-Wallis test, Bonferroni procedure, statistical significance was accepted at p≤ 0.05).

Results and Discussion

Specimens were visualized by virtual sections (FIG. 1) and 3D images.

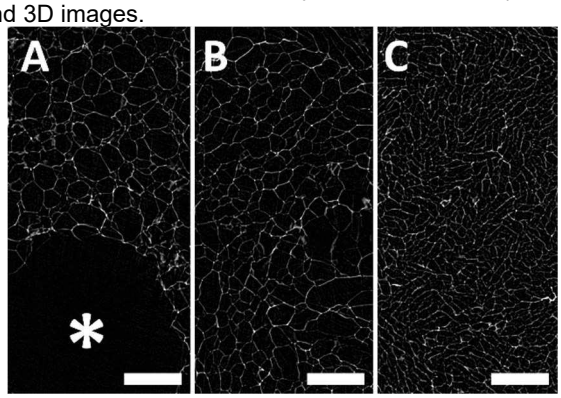

FIG. 1. Micro-CT 2D visualizations: A) -30°C; B) -80°C; C) -190°C. Differences in structure are clearly visible; large pore is shown in A(*). Scale-bars = 500 μm.

Pore size (Structure separation) was significantly reduced with lower temperature as was expected (FIG. 2-left). Thickness of pore walls (Structure thickness) was highest in -30°C (16.6 μm), both groups -80°C and -190°C presented lower values (approx. 16.1 μm). Porosity presented the highest values in -80°C (FIG. 2-right). Since closed porosity was below 0.01% in all specimens, open porosity was considered as total porosity. Object surface density was highest in -190°C (39 mm^{-1}), both groups (-30°C, -80°C) presented lower values (approx. 22 mm^{-1}).

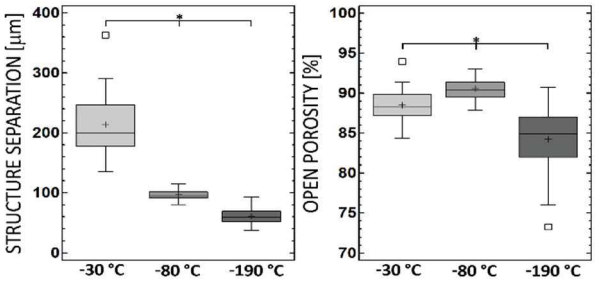

FIG. 2. Selected parameters: Structure separation (i.e. pore size) and Open porosity value in all groups. * denotes statistical significant differences, p=0.05.

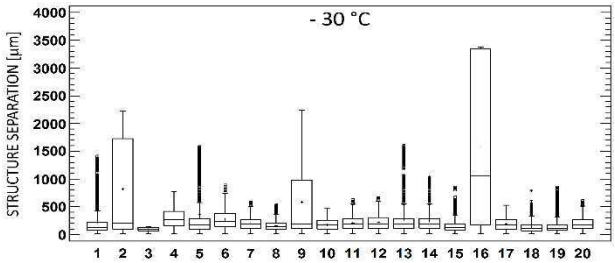

FIG. 3. Structure separation (Pore size) distribution in each specimen of -30°C group. 7 specimens present pores with diameter > 1 000 μm.

Variability of pore sizes among all specimens in each group was evaluated and significant differences were found. Variation coefficients (the ratio of the interquartile range to the median) were: -30°C=92.9%, -80°C=33.3%, -190°C=50%. Some specimens in -30°C group (FIG. 4) contained extensively large pores (see FIG. 1A*), which may limit application of these biomaterials.

Conclusions

Temperature of lyophilisation influences tissue engineering scaffolds structure. The most convenient mean pore size regarding recommended values presented -30°C group. However, due to its high variability, its application may be limited. Open porosity, important parameter, was highest in -80°C specimens. In general, the most predictable fabrication outcomes present lyophilisation at -80°C. In general, lyophilisation in -80°C was the most convenient fabrication method.

Acknowledgments

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