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ORIGINAL ARTICLE

Quantitative determination of residual 1,4-dioxane in three-dimensional printed bone scaffold

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KEYWORDS
headspace gas chromatography mass spectrometry;
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Summary *Background/Objective:* A novel porous scaffold poly (lactide-*co*-glycolide) and tricalcium phosphate (PLGA/TCP) was developed by three-dimensional printing technology for bone defect repair. As a Class 2 solvent with less severe toxicity, content of residual 1,4-dioxane in this newly developed scaffold should be rigorously controlled when it is translated to clinical use. In this study, a headspace gas chromatography-mass spectrometric (HS-GC-MS) method and related testing protocol were developed for quantitative determination of 1,4-dioxane in the PLGA/TCP composite scaffolds.
Methods: Matrix effect analysis was used to optimise the pretreatment method of the scaffolds. Then, the procedure for testing 1,4-dioxane using HS-GC-MS was set up. The accuracy, precision, and robustness of this newly developed quantitative method were also validated before quantification of 1,4-dioxane in the scaffolds with different drying procedures.
Results: Dimethyl formamide (DMF) was the optimal solvent for dissolving scaffolds for GC-MS with proper sensitivity and without matrix effect. Then, the optimised procedure was determined as: the scaffolds were dissolved in DMF and kept at 90°C for 40 minutes, separated on a HP-5MS column, and detected by mass spectroscopy. Recovery experiments

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gave 97.9–100.7% recovery for 1,4-dioxane. The linear range for 1,4-dioxane was determined as 1–40 ppm with liner correlation coefficient ≥ 0.9999. Intraday and interday precision was determined as being within relative standard deviation of below 0.68%. The passable drying procedure was related to lyophilising (−50°C, 50 Pa) the scaffolds for 2 days and drying in vacuum (50 Pa) for 7 days.

Conclusion: This is the first quantitative method established to test 1,4-dixoane in a novel scaffold. This method was validated with good accuracy and reproducibility, and met the methodological requirements of the Guideline 9101 documented in the Chinese Pharmacopoeia 2015 Edition.

The translational potential of this article: This quantitative method for determination of residual 1,4-dioxane in the novel scaffolds is a key technical method during its translation into clinical use because this method is an important and indispensable file in the enterprise standard when the porous scaffold is registered as a Class III implanted medical device for bone defect repair, which is used to guarantee the safety of the scaffolds. It is also applied to optimise the drying process of scaffolds and to monitor the quality of scaffolds in the industrialisation process. Further, this method provides references for other solvents quantitative determination in porous scaffolds or materials.

Introduction

Steroid-associated osteonecrosis (SAON) is one of the most difficult diseases as it largely occurs in large joints leading to articular surface collapse and expensive joint replacement [1–3]. The prognosis of joint replacement in SAON patients is poor due to osteolysis and impaired osteogenesis [4,5]. Accordingly, research and development of osteopromotive scaffolds ready for implantation, which would be capable of activating host cells to differentiate into angiogenic and osteogenic lineage, would be desirable [6,7].

Low-temperature deposition manufacturing (LDM) is a unique rapid prototype technology [8,9] that provides accurate point-to-point control of liquid moulding materials to form scaffolds with a gradient pore structure [10,11]. In some studies, series of porous poly(lactic-*co*-glycolic acid) and tricalcium phosphate (PLGA/TCP) scaffold were fabricated using the LDM technique [12]. The PLGA/TCP scaffolds exhibited osteoconductive activity and could also be used as a drug carrier [11,13–20].

In the process of scaffold fabrication, 1,4-dioxane was optimised as a necessary solvent used for dissolving PLGA due to its low melting point, liposolubility and highly volatile nature. However, with two oxygen atoms, 1,4-dioxane has been shown to be carcinogenic to animals and humans [21–24]. Furthermore, 1,4-dioxane may also be toxic to liver, lungs, kidneys, and the central nervous system [25]. 1,4-dioxane was defined as a Class 2 solvent associated with less severe toxicity, which should be limited to below 380 ppm according to international regulations, such as Chinese Pharmacopoeia [26], International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [27], and United States Pharmacopoeia [28]. Therefore, it is mandatory to test residual 1,4-dioxane for PLGA/TCP scaffolds, which is also necessary for registration and quality control of novel scaffolds.

To date, established testing methods for 1,4-dioxane have been used for quantification analysis in liquid or liquid-like samples, such as drinking water, waste water, vaccines, and cosmetics. These samples were usually processed with double distilled water [29,30]. For scaffolds, the pretreatment is a necessary step to select a proper solvent before analysis.

It was reported that 1,4-dioxane was determined reliably in water by various techniques including direct aqueous injection, purge and trap gas chromatography-mass spectrometric (GC-MS), and GC-MS analysis of continuous liquid–liquid extraction extracts [30]. Conventional purge and trap GC-MS is strictly limited by poor purge efficiency of 1,4-dioxane whose detection limit is about 100 times higher than those efficiently purged volatile organic compounds [30]. Headspace GC-MS (HS-GC-MS) was used commonly in the determination of poorly purged organic solvents in cosmetics [31] as it could reach the target compound to eliminate the background interference in special boiling point of volatile organic compounds. Therefore, HS-GC-MS was chosen as an analysis method for scaffolds to simplify the pretreatment process and acquire high quantity chromatogram or spectrum.

The purpose of this investigation was to develop a selective, rapid, yet simple and robust method for the determination of 1,4-dioxane in PLGA/TCP porous scaffolds by HS-GC-MS. The procedure included pretreatment of the scaffolds, sample enrichment by headspace apparatus, separation from other components by GC, and identification by MS. The method was validated and applied to optimise the drying process of scaffolds during fabrication that could also be generalised as standard and/or guideline for wide applications.

Experimental

Standards and reagents

1,4-dioxane (99.9%, GC grade) and dimethyl sulphoxide (GC grade) were obtained from Aladdin (Shanghai, China).

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