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Deformation analysis of gellan-gum based bone scaffold using on-the-fly tomography

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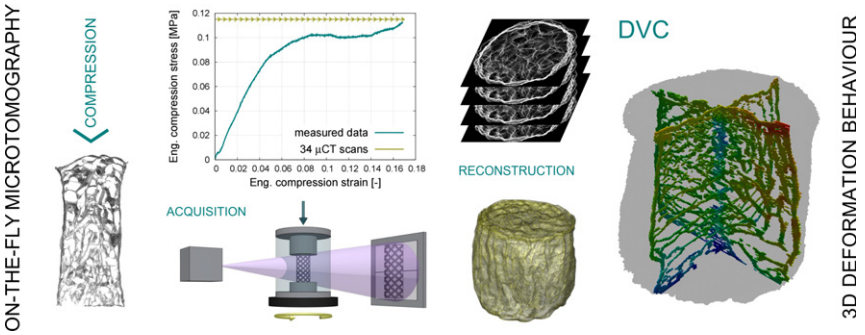
HIGHLIGHTS

- Volumetric compressive characteristics of a gellan-gum bioactive-glass scaffold for bone tissue engineering are assessed.
- Deforming microstructure of the scaffold is observed during on-the-fly X-ray micro-tomography under loading.
- In-house micro-tomographical setup equipped with loading device is used to develop voxel model of deforming microstructure.
- High-resolution displacement and strain fields are evaluated using custom digital volume correlation method.
- Reliability and ability of proposed method to capture the deformation of microstructure with high confidence are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Porous hydrogel-based structures reinforced by bioactive nano-particles allows one to design scaffolds with controlled stiffness, strength, and permeability for bone-tissue engineering applications. To be able to reliably assess the mechanical properties, it is necessary to study the material's deformation response on a volumetric basis and in high detail. In this paper, we present an investigation on the compressive characteristics of highly-relaxing gellan-gum bioactive-glass scaffold subjected to continuous uniaxial quasi-static compression. The sample was compressed with a loading rate of $0.4\,\mu m \cdot s^{-1}$ and simultaneously irradiated by X-rays during several micro-tomographical scans to obtain data for the evaluation of the deformation and strain fields using digital volume correlation (DVC). Such DVC evaluated on-the-fly micro-tomography was very challenging due to the low thickness of cell-walls and the material's intrinsic low attenuation

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of X-rays. Thus, we employed loading and tomographical devices equipped with a single-photon counting detector coupled with a DVC procedure, all developed in-house. From the acquired 34 tomographical scans, high-resolution voxel models with a resolution of 29.77 μm were developed and subjected to DVC to obtain detailed deformation and strain fields of the material. It is shown that the presented method is suitable for the precise determination of the deformation response of the predominantly organic material developed as a biocompatible, bioresorbable bone scaffold.

1. Introduction

To overcome problems encountered in the usage of autografts and allografts (i.e., donor site morbidity, loss of bone inductive factors, resorption during healing [1]) artificial bones represent an attractive alternative with significant potential. Bone tissue engineering and specifically designed implants with functionally graded properties represent a modern approach to the bone repair process [2], where the goal is an implant that is anatomically and functionally compatible with the surrounding tissue [3]. For these reasons, preferably, the bioresorbable and bioactive materials with the potential to support and stimulate the regeneration of the natural tissue should be used [4].

Metallic biomaterials are an established group of materials used primarily for load bearing applications such as joint implants and fixations of bone fractures, where the attention is currently, particularly, paid to the development of new processing techniques, for instance the utilisation of additive manufacturing, which is being considered for the production of e.g., poly-lactic acid implants [5] or selective laser sintering based processing of titanium alloys [6–8]. However, being a specific sub-group of biomaterials, bone-scaffold materials are typically divided into three main groups: i) organic, ii) inorganic, and iii) composite materials. Several different artificial polymers including various forms of poly-lactides (PLAs) and natural polymers, such as proteins and polysaccharides, can be assigned to the organic group. PLAs are currently, commonly, used in orthopaedic surgery, oral and maxillofacial surgery and exhibit very good mechanical compatibility with natural tissue together with biodegradation via hydrolysis by the bulk erosion process [9]. Among the natural polymers, the polysaccharide gellan gum (GG) has been introduced as an intrinsically biocompatible, non-cytotoxic polymer for tissue engineering applications [10,11]. Its advantages can be seen in the adjustability of microstructure-dependent mechanical characteristics and the ability for in-situ gelation. Inorganic materials have been extensively developed for the purpose of hard-tissue regeneration using hydroxyapatite (HAp), tri-calcium phosphate (TCP), and bioactive-glass (BAG) scaffolds [12,13]. HAp and TCP offer a chemical composition similar to the natural bone, which renders them popular in surgical practice. However, use of HAp also brings risk in a possible inflammatory response after implantation (e.g., [14,15]). For this reason, BAGs have attracted a significant amount of attention thanks to their full resorbability and potential to increase solubility. Furthermore, BAG induces HAp precipitation in the presence of biological fluids leading to the enhanced mineralisation of bone tissue. The most notable disadvantage of inorganic materials are generally unfavourable mechanical properties - stiffness incompatibility in case of HAp, TCP and intrinsic brittleness, a negligible elastic region and high hardness of BAG.

Recently, bone scaffolds manufactured using bioactive substances and cells have recently been accepted as the modern basis for tissue regeneration strategy for both hard- and soft-tissue engineering [16,17]. To solve individual disadvantages of the GG and BAG materials, i.e., to improve the mechanical properties and increase the bioactivity of GG, nanoparticulate BAG can be used to form a composite material with an extended application range, including bone repairs also. It has been shown that such a GG-BAG hydrogel is suitable for the bone scaffold production and offers several advantages including biodegradability, the possibility to mineralise in-vitro and the possible combination with adipose stem cells for both cellular and acellular regeneration strategies [18]. The fabricated material forms a composite combining favourable properties of both organic and inorganic materials in a synergistic manner capable of forming an apatite layer, when exposed to bodily fluids, and to ensure adherence of human-adipose derived stem-cells (hASC) that importantly remain viable [10,18]. Nevertheless, the morphological modification of bulk GG-BAG, resulting in a closed-pore foam-like material satisfies the requirements on biodegradability and bioactivity only, while the effective stiffness of such structures is significantly lower than the stiffness of the natural bone. This can be overcome by a combination of directly controlled microstructures of the scaffold (i.e., by utilising methods of additive manufacturing) and a bioresorbable/bioactive substance, such as GG-BAG, that either forms the entire scaffold's microstructure or overlays its surface. This technique opens possibilities for extensive optimisation of several other important characteristics also, including gradients in mechanical properties to minimise the stress-shielding effects and the modification of the scaffold's permeability characteristics to conform with the surrounding natural bone [19]. Both of these characteristics are crucial for the success of the implantation as they enable the occurrence of cell adhesion within a few hours after implantation and greatly influence the formation of intercellular signalling pathways that direct cell viability, proliferation and differentiation [20,21].

Currently, the only available method for the assessment of the volumetric deformation behaviour on a microscale is time-lapse micro-tomographical imaging (micro-CT) under loading. To achieve this, a special loading device is directly placed in the micro-CT scanner [22]. The sample is placed in an environmental chamber enabling one to test the samples, also under simulated biological conditions. Commonly, loading is applied to the samples in discrete steps and tomographical imaging is performed in between individual loading steps. Individual deforming microstructural elements within the sample's microstructure can be then captured with high confidence using the imaging chain, preferably composed of a micro-focus X-ray source with micrometric spot size coupled with a single photon counting detector. Moreover, digital volume correlation (DVC) can be used to quantify the displacement and strain fields in the microstructure [23]. The limitations of the conventional time-lapse micro-CT measurement of the GG-BAG material primarily consist of the performance of the used imaging chain. The period of time between the individual loading steps is given by the attenuation characteristics of the investigated material, the parameters of the X-ray source, the sensitivity/efficiency of the detector and its readout speed. Due to the natural relaxation effect, whose magnitude may be very high in the case of porous solids manufactured from relatively compliant base materials, the applied load may significantly decrease or even reach zero level between the individual tomographical scans. Such an experiment is then, in fact, a measurement of the material's response to quasi-cyclic loading. Although the measurement in the whole range of the deformation up to