Transactions on Additive Manufacturing Meets Medicine
Trans. AMMM, Vol 1, No 1, 2019, Article ID S07T03

Infinite Science | Publishing

3D printing of smart materials for bone regeneration

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Abstract: Additive manufacturing (AM) of medical implants and scaffolds continues to receive broad attention in regenerative medicine and tissue engineering. The application of smart materials combined with AM represents a new approach to enhance the functionality of modern implants to tailor patient-specific therapies. Considering bone to be a smart materialf, this study aims to applicate barium titanate ($BaTiO_3$) as a smart, piezoelectric bone-mimicking material for AM. We present a customized powder-based 3D printing process to manufacture cylindrical, porous scaffolds. Subsequent, the scaffolds were characterised in terms of shrinkage, and cytotoxicity. The results represent the first step for the fabrication of piezoelectric barium titanate scaffolds via 3D printing.

I. Introduction

Smart materials are of increasing interest in regenerative medicine, especially for the development of responsive scaffolds with improved biocompatibility. In this context, "smart" refers to a material, which is capable of reacting on external surrounding conditions by reversibly modifying its properties [1]. One of the most relevant target tissues in clinical practice is bone. Bone is considered to be a smart material, possessing the ability to react on different kinds of stimuli, either being of mechanical (e.g. stress), physical (e.g. electrical) or chemical (e.g. growth factors) origin. Especially piezoelectric properties seem to play an important role in the bone remodelling process. Considering bone to be piezoelectric, the approach of engineering new biomaterials to mimic the piezoelectric properties of bone seems promising [2]. In this study, we aim to combine a smart, piezoelectric material ($BaTiO_3$) with an additive manufacturing process to fabricate bone mimicking scaffolds with enhanced osteoconductive and osteoinductive properties.

II. Material and methods

A powder compound of 40 Vol.-% $BaTiO_3$ (Sigma Aldrich GmbH, Taufkirchen, Germany), 40 Vol.-% HA (Friedrich Baur BioMed Center, Bayreuth, Germany) and 20 Vol.-% Polyethylmethacrylate (Degacryl 6582F, Evonik GmbH, Essen, Germany) was mixed. Subsequently cylindrical specimens and structured scaffolds were printed, utilizing a powder 3D printer VX500 (Voxeljet AG, Friedberg, Germany) [3]. The specimens were heat treated in a debinding (500° C, 90 min) and sintering (1320 °C, 4h) furnace. Due to the loss of polymeric matrix, the material composition changes towards a 50:50 ratio during the process regarding its ceramic components. Therefore, the samples are called $BaTiO_3$ (50:50) in the following. Specimens were characterized in regard of shrinkage by measuring the geometry in different directions of space (digital caliper according to DIN 862) investigating the shrinkage in diameter (δ_{ds}),height (δ_{hs}) and in volume (δ_{vs}). To assess the materials in terms of their *in vitro* biocompatibility, indirect cytotoxicity tests were performed using the eluate-exposure method (in regard to ISO 10993-5). Sintered $BaTiO_3$ (50:50) scaffolds were sterilized using dry air sterilization at 160°C for two hours. The scaffolds were immersed in cell culture medium (0.2 g / ml) to create scaffold eluates. For all in-vitro assessments, MC3T3-E1 osteoblast-like cells were used. The eluates (1 ml) of $BaTiO_3$ (50:50) scaffolds were added to the cells. Tissue culture treated polystyrene (PS) and cell culture medium containing 6% (v/v) DMSO served as positive and negative controls, respectively. The cells were incubated for 24 hours with scaffold eluates and positive and negative controls prior to analysis. To investigate cell viability and proliferation, different in-vitro characterization methods were performed. Water-soluble tetrazolium salt assay (WST-8, Cell Counting Kit-8, Sigma Aldrich, Germany) was performed to assess cell viability. Lactate dehydrogenase (LDH) levels were measured using a lactic dehydrogenase based *in vitro* toxicology assay kit (TOX7, Sigma Aldrich, Germany). Calcein acetoxymethyl ester Calcein AM and propidium iodide (PI) (both Invitrogen, USA) stainings were performed on MC3T3-E1 cells indicating live and dead cells respectively. Images of the cells after incubation were taken using an inverse fluorescence microscope (Scope.A1, Carl Zeiss, Germany). Direct cell-material interaction was assessed by seeding 100.000 cells.ml^{-1} on $BaTiO_3$ composite scaffolds followed by incubation for 24 hours. Next, cells were fixed using paraformaldehyde and glutaraldehyde and dehydrated using a gradient ethanol series as previously described [4]. The samples were critical point dried (EM CPD300, Leica, Germany) and imaged using scanning electron microscopy using a Auriga CrossBeam unit (Carl Zeiss, Germany).

DOI: 10.18416/AMMM.2019.1909S07T03

III. Results and discussion

Various 3D structured and interconnected scaffolds were successfully printed and exemplarily depicted in Fig. 1.

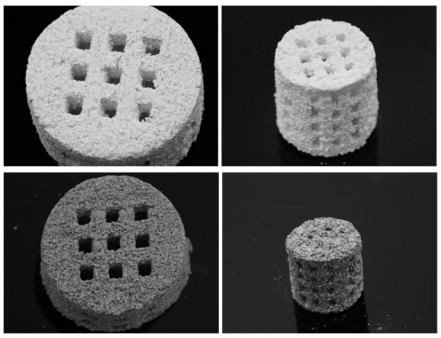

Figure 1: Interconnected porous scaffolds of BatiO₃-composite (50:50) after 3D printing (top) and sintering (bottom.)

After sintering the scaffolds showed a clear change in color, shifting from white to brown, and a change in the microstructure.

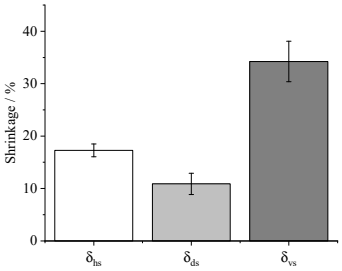

Figure 2: Influence of the sintering in terms of linear shrinkage in different directions and in volume.

Sintering resulted in an obvious shrinkage, illustrated in Fig. 2, of 34 % in volume (δ_{vs}). Moreover the scaffolds faced an anisotropic shrinkage, with a slightly increased δ_{hs} compared to δ_{ds}. Overall, the sintered specimen allowed easy handling, but showed high brittleness, indicating the need of an adjustment of the post thermal treatment.

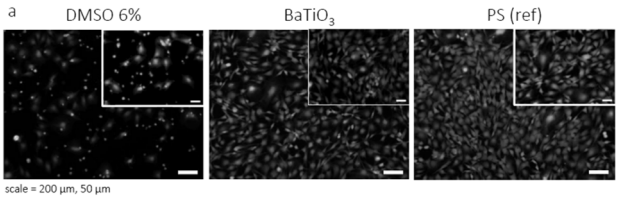

Figure 3: LIVE/DEAD stainings of MC3T3-E1 cells cultured in eluates of $BaTiO_3$, DMSO 6% (negative ctrl) and cell culture medium (PS, positive ctrl).

Live and dead staining revealed a significant difference of $BaTiO_3$ eluate compared to the negative control of DMSO 6%. Compared to the positive control, the eluated indicated no cytotoxic effect of the eluates on the cells. According to the LDH assay, no significant difference in total amount of LDH comparing PS and $BaTiO_3$ (50:50) was observed. Viability assessment by WST-8 revealed a significant difference between BaTiO3 (50:50) and the positive reference ($p < 0.05$), indicating a potentially reduced cell activity when cultured in the eluates. Both, WST-8 and LDH assay results are depicted in Fig. 4.

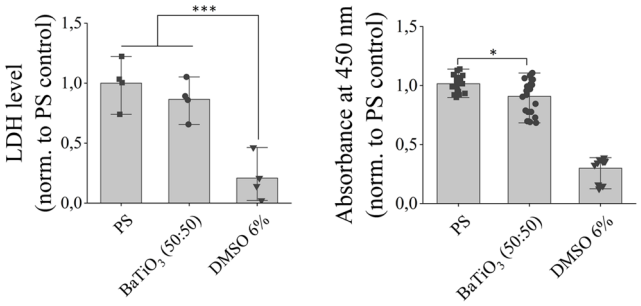

Figure 4: WST-8- and LDH assay results comparing $BaTiO_3$ eluate with negative and positive controls.

Fig. 5 depicts cells cultured on BaTiO3-composite samples. The results show significant attachment and

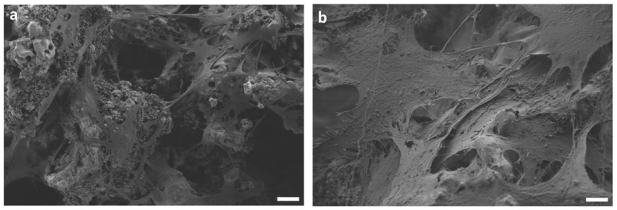

Figure 5: Scanning electron microscopy images of MC3T3 cells spreading on BaTiO3-composite (50:50) samples. Scale bars: (a) 20 µm, (b) 4 µm.

spreading of the cells on the sintered scaffolds, depicting cellular adhesion and interaction with the $BaTiO_3$ samples. These preliminary cytotoxicity screening results indicate a biocompatible material, in agreement with previous results in the literature [2].

IV. Conclusions

This work represents the first steps in the development by AM of smart, piezoelectric scaffolds for bone regeneration. The scaffolds exhibited biocompatibility and suitable cellular response. We aim to investigate the process and the material further, to enhance the mechanical and piezoelectric properties of this promising material platform.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from the German Research Foundation (DFG) by the CRC 1270 ELAINE.

AUTHOR'S STATEMENT

Research funding: The Research is supported by the German Research Foundation (DFG) Conflict of interest: Authors state no conflict of interest.

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