Injury, Int. J. Care Injured 47 S1 (2016) S52–S61

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Effects of macroporous, strontium loaded xerogel-scaffolds on new bone formation in critical-size metaphyseal fracture defects in ovariectomized rats

Seemun Ray[a,†], Ulrich Thormann[a,b,†], Ursula Sommer[a], Thaqif El Khassawna[a], Marvin Hundgeburth[a], Anja Henß[c], Marcus Rohnke[c], Katrin S. Lips[a], Christian Heiss[a,b], Sascha Heinemann[d], Thomas Hanke[d], Lutz Dürselen[e], Reinhard Schnettler[a,b], Volker Alt[a,b,*]

[a] Laboratory of Experimental Trauma Surgery, Justus-Liebig-University, Giessen, Germany
[b] Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[c] Institute of Physical Chemistry, Justus-Liebig-University Giessen, Giessen, Germany
[d] Max-Bergmann-Center of Biomaterials, Institute of Materials Science, Technische Universität Dresden, Dresden, Germany
[e] Institute for Trauma Surgery Research and Biomechanics, Centre for Musculoskeletal Research Ulm, Germany

KEYWORDS

metaphysis
fracture defect
biomaterial
scaffold
strontium
osteoporosis

ABSTRACT

New bone formation was studied in a metaphyseal fracture-defect in ovariectomized rats stimulated by a plain and a strontium-enriched macroporous silica/collagen scaffold (ScB30 and ScB30Sr20) and a compact silica/collagen xerogel (B30). 45 female Sprague-Dawley rats were randomly assigned to three different treatment groups: (1) ScB30 (n=15), (2) ScB30Sr20 (n=15), and (3) B30 (n=15). 12 weeks after bilateral ovariectomy and multi-deficient diet, a 4 mm wedge-shaped fracture-defect was created at the metaphyseal area of the left femur. A 7-hole T-shaped plate at the lateral aspect of the femur stabilized the bone and the defect was filled with ScB30, ScB30Sr20 or B30 subsequently. After six weeks, histomorphometrical analysis revealed a statistically significant higher bone volume/tissue volume ratio in the ScB30Sr20 group compared to ScB30 (p=0.043) and B30 (p=0.0001) indicating an improved formation of new bone by the strontium-enriched macroporous silica/collagen scaffold. Furthermore, immunohistochemical results showed increased expression of BMP2 and OPG and a decreased RANKL expression in the ScB30Sr20 group. This was further confirmed with the gene expression analysis where an increase in prominent bone formation markers (ALP, OCN, Runx2, Col1a1 and Col10a1) was seen. No material remnants were found in the scaffold group indicating an almost complete degradation process of the biomaterials. This is confirmed by ToF-SIMS analysis that did not detect any strontium in the ScB30Sr20 group neither in the defect nor in the surrounding tissue. Taken together, this study shows the stimulating effects of strontium through increased bone formation by up regulation of osteoanabolic markers. This work also indicates the importance of material porosity, geometry and biodegradability in bone healing.

Introduction

As one implication of over aging societies in industrial countries, osteoporosis is an increasing disease with high costs for social systems. Even low energy trauma as falls from height result in complex fractures with bony defects mainly located in the metaphyseal area of the long bones. To investigate new bone substitute materials for this specific application, it is necessary to reliably simulate the clinical fracture defect situation in osteoporotic patients. We recently established a clinically relevant metaphyseal fracture defect model in ovariectomized rats with osteopenic bone status in which different biomaterials can be tested [1]. In the next step, we showed that strontium (II) modified calcium phosphate cement can stimulate bone healing in this model compared to plain calcium phosphate cement [2].

Bio-inspired nanocomposites based on the silicification of collagen constitute a comparably young class of biomaterials [3]. Nevertheless, the chemical and technological flexibility of the silica/collagen system led to a remarkably wide range of biomaterials individually adapted for specific applications [3]. These range from soft materials like hydrogels [4] or macroporous scaffolds [5] for tissue engineering applications to compact xerogels [6] for load-bearing bone substitution applications. All these materials meet the requirements of various biomedical applications since silica and collagen turned out to be suitable partners for composite formation, resulting in advantageous synergistic effects in material properties.

* Corresponding author at: Department of Trauma Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Rudolf-Buchheim-Str. 7, 35385 Giessen, Germany. Tel. +49(0)641 985 44 601; Fax: +49 (0)641 985 44 609.
E-mail address: volker.alt@chiru.med.uni-giessen.de (V. Alt).
† Shared first co-authorship as both authors contributed equally to this work.

0020-1383/

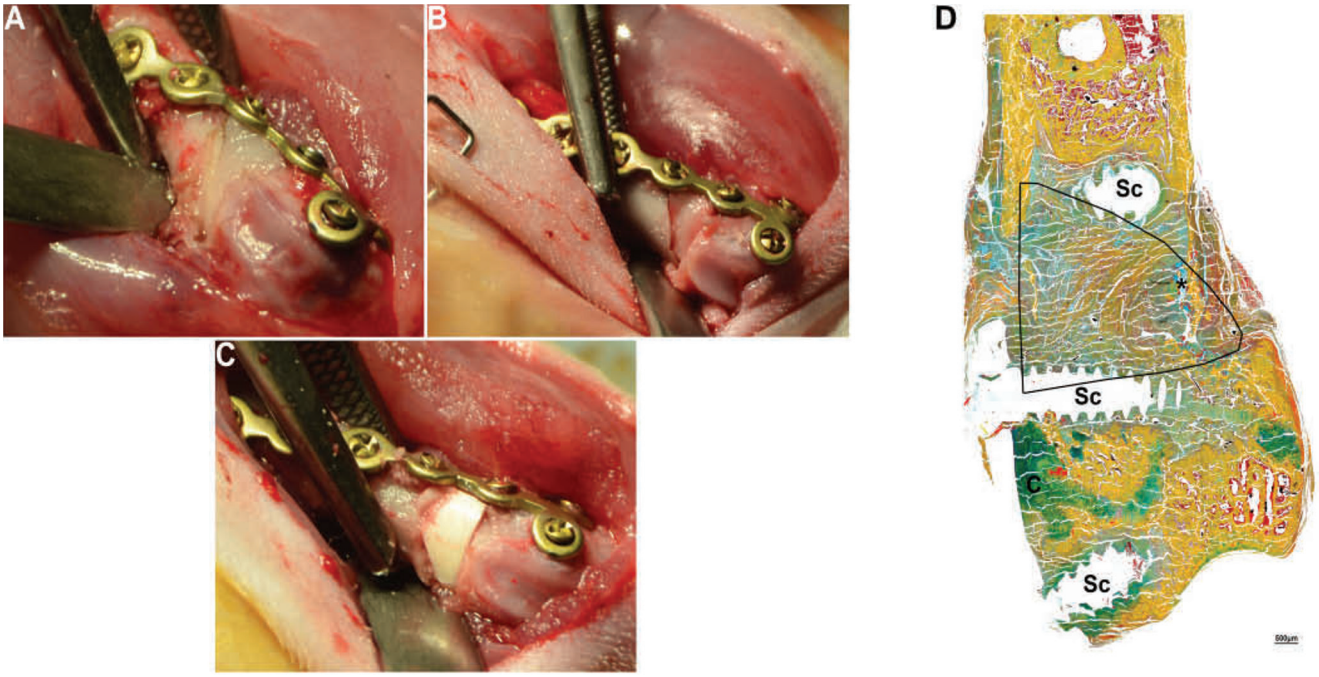

Fig. 1. Fracture-defect placed at the distal metaphysis of the left femur. The femur was stabilized with a T-shaped mini-plate and filled withScB30 (A), ScB30Sr20 (B), and B30 (C). The materials were precisely shaped to the triangular defect site with a basis length of 4 mm. They were inserted press-fit. (D) Schematic diagram for quantitative histomorphometric evaluation of movat pentachrome stained undecalcified technovit section (ScB30Sr20) indicating the region of interest (ROI). Black lines show the original defect region to study the effect of the biomaterial. Specific regions are labeled as follows: B, bone; C, cartilage; Sc, screw. * = material.

Strontium has been shown to have a two-fold action on bone formation, enhancing bone forming osteoblasts on one hand and inhibiting bone resorbing osteoclasts on the other [7,8]. Studies on healthy animals also show local administration of strontium, in the form of strontium ranelate stands better in relation to the oral administration [9,10]. Systems that aid the local release of strontium include: strontium substituted hydroxyapatites [11,12], surface modified metal oxide layers with strontium salts [13], strontium containing titanium implants [14,15], strontium loaded nanotube arrays [16,17] and strontium substituted bioactive glasses [18].

We recently developed a compact xerogel and a macroporous scaffold based on a two-phase construction of silica and collagen [5,6,19]. Additionally, we loaded the scaffold with strontium to enhance bone formation. The intention of this work is to analyze new bone formation processes in the above-described animal model induced by macroporous scaffolds based on a silica/collagen matrix as well as strontium-modified scaffolds. Therefore, the first intention of the current work is to determine the effects on bone formation in presence of a strontium-modified scaffold (ScB30Sr20) in comparison to a strontium-free scaffold (ScB30), and a compact xerogel (B30) in the above-mentioned clinically relevant fracture-defect model [1]. Second aim is the detection of the released strontium in the surrounding tissue by time of flight secondary ion mass spectrometry [ToF-SIMS] analysis and the investigation of the degradation process of the biomaterials.

Materials and methods

Ethics statement and animal study

After approval of the animal application by the local authorities according to the Protection of Animals Act (Reference number: V 54 – 19 c 20-15 (1) GI 20/28 Nr. 108/2011), 45 female Sprague-Dawley rats were used: 8 for histological and ToF-SIMS analysis and 7 for molecular analysis (per group). Each rat was randomly assigned to three different treatment groups: (1) silica/collagen xerogel scaffold (ScB30) (n=15), (2) strontium-modified silica/collagen xerogel scaffold (ScB30Sr20) (n=15), and (3) silica/collagen compact xerogel (B30) (n=15). First, we induced an osteopenic bone status by bilateral ovariectomy combined with a multi-deficient diet as described previously [1]. Briefly, under general anesthesia with intraperitoneal injection of ketamine (62.5 mg/kg bodyweight, Hostaket®, Hoechst) and xylazine (7.5 mg/kg bodyweight, Rompun®, Bayer) bilateral ovariectomy was performed. The animals were allowed two weeks of recovery and were then fed with calcium-, phosphorus- and vitamin D3-, soy- and phytoestrogen-free diet (10 mm pellets, Altromin-C1034, Altromin Spezial futter GmbH, Lage, Germany) for three months. Bone status was ensured through measurement of bone mineral density (BMD g/cm^2) by means of Dual-energy X-ray absorptiometry (DXA) using DXA scanner (Lunar prodigy, GE Healthcare, Germany). Subsequently a 4 mm defect in the distal femur metaphysis was generated, stabilized with a T-shaped mini-plate [1]. The defect was filled either with ScB30, ScB30Sr20, or B30 (Fig. 1). After six weeks, femurs were harvested for detailed investigations. In case of plate fixation failure, e.g. breakage or loosening, specimens were not taken to further analysis.

Preparation of compact xerogels and macroporous scaffolds

Compact silica/collagen xerogels were prepared as follows. The collagenous component was prepared by dialysis (MWCO 12–14 kDa, Roth, Germany) of bovine tropocollagen type I (GfN, Germany) against deionized water followed by fibrillation in 30 mM neutral sodium phosphate buffer solution, lyophilisation (Christ Alpha 1-4 laboratory freeze-dryer, Germany), and resuspension in 0.1 M TrisHCl pH 7.4 (Roth) to obtain a homogeneous 30 mg/ml suspension [6]. The silica component was prepared by 1 h hydrolysis of tetraethoxysilane (TEOS, 99%, Sigma, Germany; molar ratio TEOS/water = 1/4) under acidic conditions (0.01 M HCl) to obtain silicic acid followed by cooling in a fridge. In the