S53P4 bioactive glass and fibrin glue for the treatment of osteochondral lesions of the knee – a preliminary in vivo study in rabbits

Ancuța Marilena Zazgyva1), Simona Gurzu2), Ioan Jung2), Örs Nagy3), Gheorghe Mühlfay4), Tudor Sorin Pop3)

1)Department of Cell and Molecular Biology, University of Medicine and Pharmacy of Tîrgu Mureș, Romania
2)Department of Pathology, University of Medicine and Pharmacy of Tîrgu Mureș, Romania
3)Department of Orthopedics and Traumatology, University of Medicine and Pharmacy of Tîrgu Mureș, Romania
4)Department of Otorhinolaryngology, University of Medicine and Pharmacy of Tîrgu Mureș, Romania

Abstract
The role of the subchondral bone and the importance of treating both bone and cartilage in cases of chondral and osteochondral lesions of the knee have been highly emphasized. There are no current studies on the experimental use of bioactive glass S53P4 (BonAlive®) as granules in the treatment of osteochondral lesions of the knee. Our preliminary study was designed to establish an experimental model and assess the effect of glass granules fixed with fibrin compared to fibrin alone as fillers of the osteochondral defects created in the weight-bearing and partial weight-bearing regions of the distal femur in six adult rabbits. We found that the size of the distal femur in adult domestic rabbits allows the creation of 4 mm diameter and 5 mm deep osteochondral defects on both the medial femoral condyle and the trochlea, bilaterally, without significantly affecting the activity level of the animals. Retention of the glass granules in the defects was achieved successfully using a commercially available fibrin sealant. At five weeks post-implantation, we found macroscopic and microscopic differences between the four types of defects. The use of bioactive glass S53P4 for filling condylar osteochondral defects in rabbit femora led to the initiation of an early bone repair process, observed at five weeks after implantation, while the filling of trochlear defects with fibrin glue resulted in the appearance of cartilaginous tissue characteristic of endochondral ossification.

Keywords: osteochondral defect, bioactive glass, fibrin sealant, weight bearing.

Introduction
Articular cartilage lesions of the large joints (especially the knee) are common, but difficult to treat. In addition, a large proportion of these cases are diagnosed in young patients and can progress to major arthritic changes, with significant implications in terms of quality of life and work capacity of the patients, and thus important economic consequences. Considering the hyaline cartilage’s very limited ability to heal, finding treatment options that can achieve optimal results is of paramount importance. Many recent studies have emphasized the role of the subchondral bone in the proper functioning of articular cartilage, highlighting the need for simultaneous treatment of both components – bone and cartilage – in cases of chondral and osteochondral lesions of the knee [1–7].

From the many available bone graft substitutes [8], ceramic-based grafts, and particularly bioactive glasses, stand out as substances with special properties and important clinical implications [9]. Bioactive glasses have anti-inflammatory and antibacterial properties, as well as proven osteoconductivity [10]. Their activity is based on the fact that these absorbable materials form a layer of bioactive hydroxyapatite and release ions that stimulate cell differentiation, bone formation and the production of angiogenic growth factors [11, 12]. Bioactive glass S53P4 (commercially available as BonAlive®) reacts similarly in soft connective tissue and bone, by forming strong bonds with the collagen fibers, even in the absence of cells [13]. Starting with the assumption that S53P4 bioactive glass used in the treatment of osteochondral defects can accelerate the healing of the subchondral bone and overlying cartilage, we designed our experimental study to verify this hypothesis.

We currently have not found any studies to demonstrate an experimental model of using bioactive glass granules in osteochondral lesions in rabbits. Because of the need to test the possibility of retaining the granules with fibrin glue, our study represents a preliminary stage of establishing an experimental model, with the assessment of the size of defects that can be created in the trochlea and femoral condyles and determining immediate and late fixation of the glass granules. Our second aim was to evaluate the effect of glass granules fixed with fibrin compared to fibrin alone as fillers of the osteochondral defects created in the weight-bearing and partial weight-bearing regions of the distal femur in rabbits.

Materials and Methods
Animals and substances used
In the present study, six adult female domestic rabbits (Oryctolagus cuniculus) were used; the animals were obtained from the Animal Facility of the University of Medicine and Pharmacy of Tîrgu Mureș, Romania. To
minimize the number of necessary animal, bilateral defects were created on the distal femur. The study’s research protocol was reviewed and approved by our institution’s ethics committee.

S53P4 bioactive glass (BAG) is commercially available as different sized granules (BonAlive®, BonAlive Biomaterials Ltd., Turku, Finland). Given the volume of osteochondral defects that can be created on the distal femur of rabbits, the smallest size granules were chosen, which vary between 0.5 and 0.8 mm in diameter.

To fix and retain the granules within the defects, a fibrin sealant with a thrombin concentration of 500 UI/mL was used, which polymerizes and sets fast, in a matter of seconds (TISSEEL Lyo, Baxter Healthcare Ltd., Norfolk, UK).

**Operative technique**

For anesthesia and analgesia, 5 mg/kg body-weight (bw) Xylazine and 35 mg/kg bw Ketamine were administered intramuscularly, providing adequate muscle relaxation for 40–60 minutes.

Surgery was performed under anesthesia, in asepsis and antisepsis. After adequate preparation of the knee regions, a minimal skin incision of approximately 3 cm was made medial to the patella, and by lateral luxation of the patella, the distal epiphysis of the femur was exposed through knee flexion. This approach did not affect the integrity of the articular ligaments or patellar tendon, an important aspect for rapid postoperative recovery.

Two osteochondral defects of 4 mm in diameter and 5 mm in depth were made in each knee using a sterile drill – one on the loading surface of the medial femoral condyle and the second one in the middle of the trochlea. The location of the trochlear defect was identified by observing the insertion of the extensor digitorum longus tendon on the lateral femoral condyle – the tendon’s insertion level corresponds to the portion of the trochlea that is continuously in contact with the patella (Figure 1). The defects were filled as follows:

- BAG granules fixed with fibrin sealant in three animals, both trochlear and condylar defects (six defects of each type; Figure 2);
- fibrin sealant alone in three animals: six trochlear and six condylar defects.

Before relocating the patella, the knee was taken through a few cycles of flexion/extension, to verify the primary fixation of the granules. The wound was closed and the skin sutured with separate stitches.

Postoperatively radiographs of the hind limbs verified the placement of the osteochondral defects, also checking for possible intraoperative fractures of the femur.

**Postoperative treatment**

In the first postoperative days, an non-steroidal anti-inflammatory drug (Carprofen) was administered for analgesia, as well as a broad-spectrum antibiotic. Rabbits were kept in adequate shelter, with filtered air, controlled temperature and humidity (22±2°C, humidity 50±20%), a 12 hours light/dark cycle, and appropriate space, without restrictions on their mobility. The rabbits were fed a commercially available chow, with water ad libitum, and underwent a weekly clinical evaluation, to check for wound healing, joint mobility, movements and any signs of functional deficit.

**Euthanasia and histological assessment**

All animals were sacrificed at five weeks postoperatively. The method of euthanasia was chosen in accordance with the IV Annex of EU Directive 2010/63 of the European Parliament. After sedation using half the dose of Xylazine and Ketamine necessary for anesthesia, euthanasia was performed by intracardiac administration of 0.2 mL/animal of T61 (solution containing 200 mg of Embutramide, 50 mg of Mebezonium iodide and 5 mg of Tetracaine hydrochloride/mL).

Both distal femora were harvested, fixed in formalin and processed for histological assessment of the tissue filling the osteochondral defects. Decalcified specimens were stained with Hematoxylin–Eosin (HE), Van Gieson and Masson’s trichrome.

**Results**

All animals resumed normal walking and behavior from the first postoperative day. Local wound healing was uneventful, with normal joint mobility in all except one rabbit, which showed a slight functional deficit of the knee joints. There were no signs of infection in any of the animals.

Macroscopic examination of the repair tissue filling the defects showed significant differences between the four types of osteochondral defects created (trochlear and condylar, filled with BAG + fibrin sealant or fibrin sealant alone). Thus, the condylar defects that were filled with fibrin sealant alone demonstrated a more adequately filling when compared with the repair tissue found in the trochlear defects. Meanwhile, both the trochlear and condylar osteochondral defects that were treated by the combination of BAG and fibrin sealant had a macroscopically similar filling tissue (Figure 3).

On microscopic examination, the differences between the condylar and trochlear defects filled with either fibrin sealant alone or in combination with BAG were even more evident. In case of defects created on the femoral condyles, treatment with BAG + fibrin sealant produced a filling repair tissue that was quantitatively reduced, with the defect still visible at five weeks postoperatively. Significant hyperplasia of the osteoblasts and reactive histiocytic proliferation were founded around the defects, as highlighted by both HE, and Masson’s trichrome staining. Fibrollogenesis and fibrosis were also evident with Van Gieson staining, as shown in Figure 4; foreign body granulomas were identified in one of the six cases.

Condylar osteochondral defects that were filled with fibrin sealant alone presented a repair tissue that was similar to normal cartilage, but with areas of discontinuity that were replaced by a connective tissue, as seen in Figure 5 in Masson’s trichrome staining.

Filling the defects with BAG + fibrin or fibrin alone also produced repair tissues with different characteristics in the trochlear region of the femur. In case of using BAG granules fixed with fibrin sealant, the repair tissue was composed of fibrotic areas with newly formed blood vessels and small bone lamellae, devoid of osteoblasts. The latter were best visible with Van Gieson staining, as...
shown in Figure 6. When the trochlear defects were filled with fibrin sealant alone, the microscopic examination showed reactive endochondral ossification, as well as hyperplasia of residual synoviocytes (Figures 7 and 8).

Figure 1 – Intraoperative aspect of the osteochondral defects created on the trochlea (red arrow) and medial femoral condyle (black arrow). The green arrow indicates the extensor digitorum longus tendon and its insertion on the lateral femoral condyle, while the grey arrow indicates the patellar tendon that was laterally dislocated.

Figure 2 – Intraoperative fixation of bioactive glass S53P4 granules with the fibrin sealant.

Figure 3 – Macroscopic aspect of two of the harvested distal femora and the filler tissue: (A) Defects filled with fibrin sealant alone; (B) Defects filled with bioactive glass S53P4 granules and fibrin sealant.

Figure 4 – Microscopic aspect of the repair tissue in a condylar osteochondral defect filled with bioactive glass + fibrin, showing fibrosis (Van Gieson staining, ×200).

Figure 5 – Microscopic aspect of the repair tissue in a condylar osteochondral defect filled with fibrin sealant alone – connective tissue filling the defect area (Masson’s trichrome staining, ×200).

Figure 6 – Microscopic aspect of the repair tissue in a trochlear osteochondral defect filled with bioactive glass + fibrin sealant – small bone lamellae in the defect (Van Gieson staining, ×100).
Discussion

An essential aspect of osteochondral lesions is that, due to its special characteristics the hyaline cartilage of the knee has a limited capacity of spontaneous healing. Therefore, special therapeutic methods are required to provide the metabolic and cellular support necessary for the regeneration process. Given the role of the subchondral bone in normal cartilage function, BAG granules – with their known effects in bone regeneration – might be a suitable, and so far incompletely investigated treatment option in these cases. Also, it has been shown that small and medium granules stimulate the production of vascular endothelial growth factor (VEGF) by fibroblasts in vitro [14], a growth factor with an important role in angiogenesis and the health of the subchondral bone-articular cartilage complex [15]. Chen et al. have shown that the subchondral bone does not recover properly after bone marrow-stimulation techniques like microfractures or drilling, also emphasizing the importance of subchondral bone involvement in cartilage repair [16].

Rabbits are still frequently used in experimental studies on cartilage repair, although the use of larger animals has some advantages in terms of articular cartilage thickness. The rabbit is the primarily used animal model when testing new methods of treatment. Besides economic reasons, another advantage of the rabbit model is the presence of a bone structure that has a Haversian system, which cannot be found in smaller rodents [17]. A disadvantage of the rabbit models is the possibility for spontaneous repair of articular cartilage lesions, but the reports on this phenomenon were made on young animals. Therefore, by choosing adult animals these false results can be avoided.

Because of the special conformation of the rabbit femuro-tibial joint, the femur has weight-bearing and partial weight-bearing regions [17]. The significant differences in terms of weight-bearing in the rabbit knee as compared to the human knee have led us to create osteochondral defects both in the femoral trochlea (partial weight-bearing region) and the medial femoral condyle (weight-bearing region).

The results of the present study suggest that 4×5 mm osteochondral defects can be created safely on both the trochlea and the femoral condyle of the rabbit knee, bilaterally, with no major influence on the postoperative function of the hind limbs. Also, BAG granules of 0.5–0.8 mm in diameter can be properly fixed in these defects by using a commercially available fibrin sealant. In addition, we obtained mixed results when using BAG + fibrin sealant or fibrin sealant alone for filling osteochondral defects in rabbit femora.

A distinctive feature of our study was the location of the osteochondral defects – both in a weight-bearing region of the rabbit knee (medial femoral condyle) and a partial weight-bearing region (trochlea). We observed important differences in the microscopic structure of the filler tissue obtained in the four types of defects created. Thus, when BAG was used in combination with fibrin glue in the condylar and trochlear defects, although on macroscopic examination the filler tissue seemed sufficient, microscopically we found considerably less newly formed tissue in the trochlear defects. This might be explained by the presence of the BAG granules on macroscopic examination immediately after harvesting, and the fact that the granules were probably lost during processing of tissue samples and their place appeared microscopically empty. In contrast, in the condylar defects filled with BAG and fibrin we identified early signs of bone repair, with the appearance of newly formed blood vessels and small bone lamellae. We might infer that, if we were to sacrifice the animals at more than five weeks, we might have obtained a more advanced bone regeneration process.

In case of using fibrin sealant alone to fill the osteochondral defects, we also observed differences between the two locations of the defects: the condylar defects filled with BAG and fibrin we identified early signs of bone repair, with the appearance of newly formed blood vessels and small bone lamellae. We might infer that, if we were to sacrifice the animals at more than five weeks, we might have obtained a more advanced bone regeneration process.

In case of using fibrin sealant alone to fill the osteochondral defects, we also observed differences between the two locations of the defects: the condylar defects showed a process of fibrosis, while the trochlear defects gave very encouraging results, with the presence of a characteristic endochondral ossification cartilage and synovial cells in the vicinity of the joint space.

Recently, Chen et al. [18] highlighted the differences in articular cartilage and subchondral bone structure in the femoral condyle and trochlea of rabbits, also showing a
superior repair of trochlear cartilage defects in case of bone marrow-stimulation techniques, as compared to the femoral condyle. Similar results have also been published on an ovine osteochondral defect model [19]. In contrast, Huh et al. reported an irregular fibrous tissue filling the chondral defects created in the trochlea of rabbits, at 12 weeks after microfracture [20]. Therefore, it is safe to say that, although a series of previous clinical and experimental studies have indicated an influence of lesion location on articular cartilage repair outcomes, there are still discrepant results reported in the literature [18]. Despite the short duration of our study, its results are in line with some of the data published, showing a superior repair process in the trochlear region of the rabbit knee.

It is possible that the biomechanical differences between the two defect locations could explain the distinct results. It appears that the use of the fibrin sealant alone in regions of partial weight bearing (where the forces acting on the filler material are not very large) yields superior results to its combination with BAG. One might infer that the cellular elements needed for the repair process seem to prefer the conditions offered by the fibrin sealant. The situation observed in the condylar defects is somewhat reversed, with discrete signs of bone repair initiated in the presence of BAG, and only fibrosis when filled with fibrin alone. Thus, it is seems that the more resistant structure created by the glass granules is favorable in the weight-bearing region of the femur. When making these interpretations, it is essential to take into account the special biomechanics of the rabbit knee, in which the highly developed muscles of the hind limbs are responsible for the occurrence of significant forces.

A series of different studies evaluated the use of combinations of various substances for the treatment of osteochondral defects created in the rabbit knee. Tanideh et al. obtained good results using xiphoid cartilage grafts and fibrin glue [21], and recently Horňák et al. implanted chondrocytes in a fibrin sealant [22]. Also, Jang et al. reported the outcomes of using a mixture of bone marrow concentrate, hyaluronic acid and fibrin to treat trochlear chondral defects [23]. Although BAG had been used before in experimental studies on cartilage repair [24], to our knowledge this is the first time that the granular form of the substance was tested in osteochondral defects of the rabbit knee. Our study’s major shortcomings are its short duration and the fact that the cellular elements needed for the repair process seem to prefer the conditions offered by the fibrin sealant. The situation observed in the condylar defects is somewhat reversed, with discrete signs of bone repair initiated in the presence of BAG, and only fibrosis when filled with fibrin alone. Thus, it is seems that the more resistant structure created by the glass granules is favorable in the weight-bearing region of the femur. When making these interpretations, it is essential to take into account the special biomechanics of the rabbit knee, in which the highly developed muscles of the hind limbs are responsible for the occurrence of significant forces.

Although BAG had been used before in experimental studies on cartilage repair [24], to our knowledge this is the first time that the granular form of the substance was tested in osteochondral defects of the rabbit knee. Our study’s major shortcomings are its short duration and the small number of animals used. In order to address the first problem, we are currently planning a follow-up study on a larger number of animals, which will be sacrificed at 12 and 24 weeks after surgery, hopefully offering more details on the results we observed. Regarding the second aspect, because of the preliminary nature of our study, we chose to minimize the number of animals used.

Conclusions

The size of the distal femur in the adult domestic rabbit allows the creation of 4 mm diameter and 5 mm deep osteochondral defects on both the femoral condyle and the trochlea, bilaterally, without significantly affecting the activity level of the animals, thus enabling the assessment of the repair tissue formed in different loading conditions. A commercially available fibrin sealant can be successfully used to retain bioactive glass granules in the defects, offering a fast intra-operative and a subsequently stable fixation. The use of bioactive glass S53P4 for filling condylar osteochondral defects in rabbit femora led to the initiation of an early bone repair process, observed at five weeks after implantation, while the filling of trochlear defects with fibrin glue resulted in the appearance of cartilaginous tissue characteristic of endochondral ossification.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

We would like to thank the staff of the Animal Facility and Experimental Station of the University of Medicine and Pharmacy of Tîrgu Mureş for their kind help and technical support in conducting our study.

References


Corresponding author
Gheorghe Mühlfay, Associate Professor, MD, PhD, Department of Otolaryngology, University of Medicine and Pharmacy of Tîrgu Mureș, 38 Gheorghe Marinescu Street, 540139 Tîrgu Mureș, Romania; Phone +40722–363 411, Fax +40265–211 926, e-mail: ghe.muhlfay@gmail.com

Received: February 17, 2015
Accepted: November 16, 2015