Histological Study of the Effect of Bioactive Glass on Tibial

Bone Repair in Rats

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Abstract: The main goal of this study was to histologically evaluate the healing of surgically created defects on the tibiae of

adult rats after implantation of bioactive glass. Twenty adult Wistar rats (body weight of 300g) were divided into two groups:

bioglass treated group (n=10) and control group (n=10). Unicortical bone defects with 3-mm diameter were performed in both

tibiae of the animals and filled with bioglass particles. The rats were then sacrificed at 14, 30 , 60 and 90 days, and the tissues

were prepared for histological processing, sectioning, and staining with hematoxylin and eosin, as well as Mallory trichrome,

and analyzed under light microscope. Within 7-14 days, both groups presented connective tissue septa with new bone

formation, more intense in bioglass treated group. In the subsequent periods (30, 60 and 90 days), these groups presented more

mature bone tissue around the glass particles. Bone trabeculae formed in all experimental periods were juxtaposed to the glass

particles. It can be concluded that bioglass materials promoted bone formation over the entire extension of the defect,

independently of the size of the granules, thus confirming their biological osteoconductive property.

Keywords: Bioglass, Albino Rat, Tibial Repair, Bone Healing

1. Introduction

Millions of fractures occur every year worldwide, with 6.2

million of them being reported per year in the United States

(1). Among those, 5–10% show delayed healing; many

persist for more than 9 months, and thus are termed nonunion

fractures. Multiple factors can impair fracture

consolidation, including bone loss caused by diseases, trauma,

or tumor resection. Hence, there remains a need to learn more

about the biology of fracture healing as well as to develop

strategies for ensuring normal repair of the skeleton (2).

Bone graft substitutes can be broadly divided into two

main groups; biological and synthetic materials. Biological

substitutes broadly include allografts or xenografts, and also

corals (3), natural polymers – collagen like (4) or

demineralized bone matrix (DBM) (5). Allografts/xenografts

are still related with risk of disease transmission or immune

rejection, as well as with reduced biological properties

following sterilization and storage, (6), and thus have limited

usage. Synthetic graft substitutes include porous metals, (7),

bioactive glasses, (8), glass–ceramics, (9), synthetic polymers

(e.g. synthetic hydroxyapatite), (10), and calcium

phosphates/sulphates (11). Such materials pose an ever

expanding portfolio of indications and have attracted

significant scientific and clinical interest.

Calcium-phosphate ceramics, such as hydroxyapatite, have

been used because their chemical composition is closely

related to that of the mineral phase of bone (12). These

ceramics are adequately biocompatible, and do not induce

adverse local tissue reactions, immunogenicity, or systemic

toxicity. Furthermore, because this material is

osteoconductive, it acts as a support for new bone formation

within the pore sites, which are deliberately generated in the

structure, (13).

Unexpected results due to the use of the materials

mentioned above have directed technological advances to

recent studies with some bio-active materials to be used as

bone substitutes, in several kinds of defects in the field of

Dentistry. Bioactive glass has shown the ability to help bone

regeneration and clinical insertion gain, with better results

than other materials available, (14, 15, 16 &18). This

material has also demonstrated osteoconductive and

osteopromotive abilities in the biocompatible interface for

osseous migration, and a bioactive surface colonized by osteogenic cells free in the surgical wound (19).

The use of Bioglass particles promotes a much faster

proliferation of new bone tissue, comparable to that

occurring after the use of autogenous bone graft; furthermore,

the combination of Bioglass granules and autogenous bone

results in more bone growth when compared to the

autogenous material, (20). Bioglasses inducing active

biomineralization for bone regeneration have been a high

demand in the development of clinical regenerative medicine.

Recent development of biomaterials in the field of tissue

regeneration includes bioactivity inducing cell adhesion, and

differentiation to achieve early healing efficacy (21).

One of the most common and studied bioactive glasses is

Bioglass 45S5, which has been known as the bioactive glass

with the highest bioactivity index. It was first introduced in

the early 1970s by Hench (22) and since then, it has been

used in many clinical applications, including ridge

preservation, sinus augmentation, and the repair of

periodontal bone defects (23). It is a silica-based meltderived

glass characterized by a SiO2 content of less than 60%, a

high Na2O and CaO content, and a high CaO: P2O5 ratio.

Bioglass 45S5has been shown to stimulate in vitro

osteogenesis inducing proliferation and differentiation of

human fibroblasts and osteoblasts (24,25 &26).

A novel fully-crystallized bioactive glass-ceramic of the

quaternary P2O5–Na2O–CaO–SiO2 system has been

developed (Biosilicate, patent application WO 2004/ 074199).

Therefore, full crystallization of the material may lead to

enhanced mechanical properties of the bulk material or less

sharp and abrasive particles when the material is milled to a

powder. The Biosilicate has presented a stimulatory effect on

bone cell metabolism. Comparing the growth of osteogenic

cells on Biosilicate and Bioglass 45S5 disks for a period of

up to 17 days, they found that, although no significant

differences were detected in terms of protein content and

alkaline phosphatase activity at days 11 and 17, Biosilicate

supported significantly larger areas of calcified matrix at day

17. Results indicate that full crystallization of bioactive

glasses in a range of compositions of the system P2O5–

Na2O–CaO–SiO2 may promote enhancement of in vitro

bone-like tissue formation in an osteogenic cell culture

system, (27).

Notwithstanding the positive effects of Biosilicate on bone

cell proliferation, studies investigating its effects on bone

healing are fairly limited in the literature. To the best of our

knowledge, there is one study demonstrating in vitro

osteogenesis on a highly bioactive glass-ceramic. It is

important to emphasize that in vitro studies do not consider

the complex homeostatic situation that occurs in vivo. In

order to progress our understanding of the physiological

processes of the Biosilicate\_ on fracture consolidation, the

goal of Ribeiro and Masumoto (28) study was to examine the

mechanical and histological characteristics of bone defects

filled with two different particle sizes of biosilicate materials

(180–212 and 300–355 lm mean size) and to compare these

characteristics to those obtained with a Bioglass\_ material of

similar particle sizes. An additional control group, that

remained empty, was included. Recently, we have applied

this methodology with success in rats exposed to laser,

treated or not with anti-inflammatory drugs (29).

2. Material and Methods

In the present study, 20 male rats (Rattus norvegicus,

Albinus, Wistar) weighing 300g were used, which were fed a

solid diet before and during the experimental period and

received water ad libitum. They were classified into two

groups; control group (10 rats), and bioglass treated group

(10 rats).

2.1. Bone Substitutes

2.1.1. Preparation of Bioactive Silicate Glass

All chemicals used in preparation of glasses such as finegrained

Quartz (SiO2), calcium carbonate (CaCO3), sodium

carbonate (Na2CO3), and ammonium dihydrogen

orthophosphate (NH4H2PO4) are selected from high pure

chemicals. All the chemicals were of analytical grade and

were used without further purification. Quartz was used for

silica (SiO2). Lime (CaO) and soda (Na2O) were introduced

in the form of their respective anhydrous carbonates.

Phosphorus pentoxide (P2O5) was added in the form of

ammonium dihydrogen orthophosphate (NH4H2PO4) and

finally ZnO was added. The batches were weighed out and

then melted in an Pt–2% Rh crucible using electric furnace at

1500 oC for 2 h and the melts were rotated two times to

achieve homogeneity. Upon complete melting, the glasses

were cast in a preheated stainless steel rectangular mould of

the dimensions of 1 cm x 4 cm x 1 cm preheated to about 250

oC. The glass samples were transferred to an annealing

muffle furnace adjusted at 740 oC and the muffle was left to

cool slowly to room temperature. The samples were polished

with 600-grit silicon carbide until their thickness became

1mm and then polished with 1200- d 2500-grit of silicon

carbide and cerium oxide ( table1).

Table 1. Bioactive Silicate Glass Sample Composition.

SiO2

Wt%

Na2O

Wt%

CaO

Wt%

P2O5

Wt%

ZnO

Wt%

45 22.5 23.5 6 3

2.1.2. Heat-treatment (Conversion to Glass–ceramic)

The glass sample was thermally heated in two-steps

regime at the mentioned temperatures. Glass was heated

slowly to the first nucleation temperature (550 0C) for the

formation of sufficient nuclei sites and after holding for 3 h,

it was then further heated or raised to reach the second

chosen crystal growth temperature (7630C), and after a

second hold for 3 h, the specimen was left to cool inside

room temperature at a rate of 20 0C/h., (22).

2.2. Surgical Procedure

Before surgery, the animals were weighed for a correct

calculation of the anesthetic dosage. General anesthesia was

used with intramuscular application of a sedative solution - hydrochloride 2-(2.6 xylidine)-5.6-dyhidro

and ketamine anesthetic 1.0g , in the proportion

the 0.1ml/100g of body weight.

After trichotomy and asepsis of the surgical

iodine, the lateral and superior aspects of

posterior paws were exposed with a 1.5-

using a 15 interchangeable blade, on a Bard

photographed grossly, (Fig.6). Tissue

performed with periosteum elevators and a

as to obtain a mucoperiosteal flap to expose

and allow free access to create osseous defects.

(right in some and left sided in others), a monocortical

defect measuring 3mm in diameter was prepared

electric engine, using a 1/16 reduction, straight

drills, at a speed of 1500 rpm, under copious

0.9% sterile saline solution throughout the bone

Before placing bone filling glass materials,

were irrigated with antibiotic.

Bioactive glass granules were placed to fill

The materials were prepared just before

sterile saline solution was added to the flasks,

gamma rays provided by the manufacturer,

texture was obtained. The flaps were closed

intermittent sutures, and deeper planes

absorbable Vicryl 4-0 suture. Immediately

procedure, all animals received intramuscular

antibiotics and anti-inflammatory drugs,

0.2ml/kg of body weight.

The site of operation was seen via x-ray

Fig. 1. A photomicrograph of the rat tibia, 14 days

granulation tissues (gt). There are many bone trabeculae

1(2): 13-

-4H-1.3- thiazine ,

of 1:0.5ml in

field with

the tibiae in both

-cm long incision,

Bard-Parker scalpel,

separation was

Molt elevator, so

the bone tissue

In both paws

bone

with aseptic

tip, trephine

irrigation with

manipulation.

the bone cavities

the bone defect.

being used, 0.9%

sterilized with

until a paste-like

with 4-0 silk

were closed with

after the surgical

application of

in the dosage of

in fig.8. However,

the site of healing was evident in

2.3. Histological Study

The animals were sacrificed

at 14, 30, 60 and 90 days after

removal of soft tissues, bone

implanted material were obtained

formalin solution for at least 48

accomplished in 20% sodium

solution, 1:1. The decalcified

paraffin and 6-μm thick semiand

stained with hematoxylinfor

light microscopy analysis (30

3. Results

After 14 days, the spaces filled

materials showed varying sizes

granulation tissue and thin woven

the trabeculae were lamellar

marrow tissue. That aspect could

the surgical defect, extending inside

particle spaces were divided by

dividing them into smaller portions.

observed next to the defect

trabeculae covered by osteoblasts,

and inside the medullary channel

after operation (bioglass treated) showing; spaces filled with bioglass

(bt) with newly formed bone cells (bc). Masson trichrome X 200.

-21 15

fig.7.

with an overdose of anesthetic

the surgical procedures. After

fragments including the

and fixed in a 10%

hours. Decalcification was

citrate and 50% formic acid

samples were embedded in

-serial sections were obtained

-eosin and Mallory trichrome

30).

by particles of implanted

and were surrounded by

bone trabeculae. Some of

and were associated with

be seen up to the center of

the marrow space. Some

thin bone septa on the inside,

New bone formation was

borders, with newly formed

both facing the periosteum

(fig.1).

particles (bg) surrounded by