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Zinc-doped bioactive glass behavior evaluated after irradiation and in vivo assays: Antioxidative/oxidative balance

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Outline

Introduction

Preparation of bioactive glass (Zn-46S6)

Physicochemical characterization
Chemical reactivity – Bioactivity

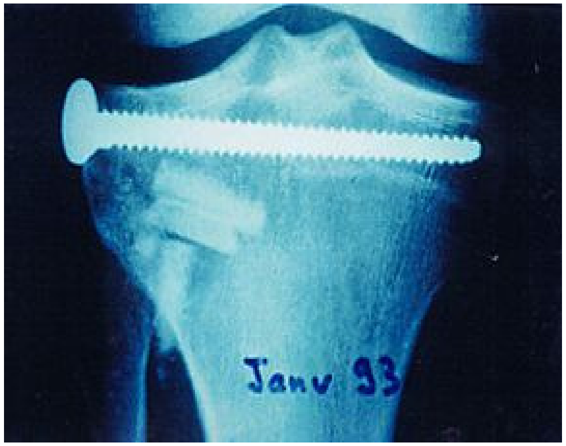
In vivo assays: Antioxidative / Oxidative balance

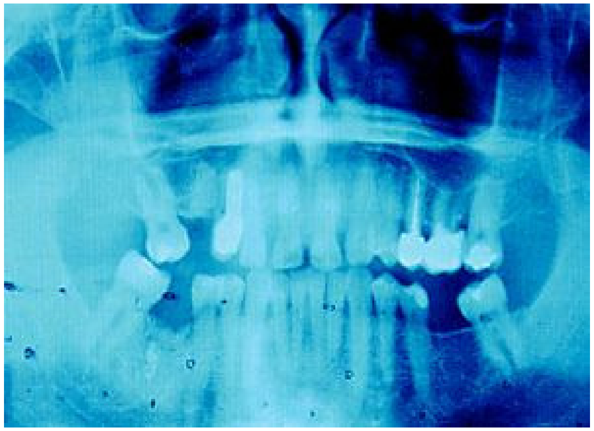
Conclusion

Context : The use of the natural grafts is progressively abandoned by the surgeons because of transmission risks of virus.

Objective : implementation of filling biomaterials:
Pure and doped

Field : Orthopedic and/or maxillo facial surgery





Synthetic biomaterials

Advantages of synthetic bioactive materials:

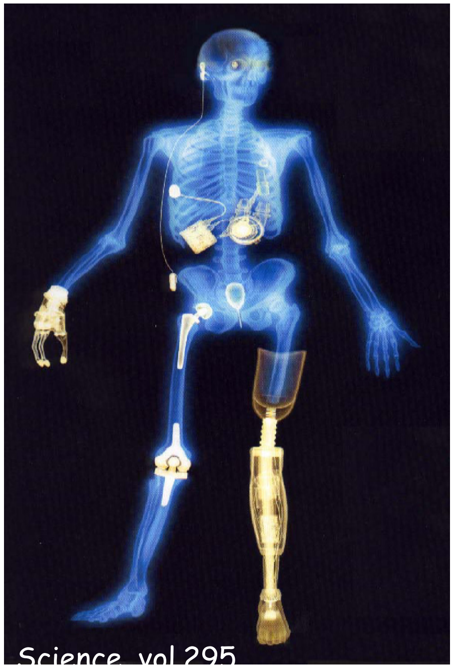
* significant potential in biomedical research field.
* facilitate bone integration of the implant,
* availability
* Association with atomic elements (Zn, Mg, Sr ....)
* Drug delivery (gentamicine, chitosan..)

The bioactive behavior of synthetic biomaterials:

*capability of bone bonding is attributed to the formation of an apatite-like layer, whose composition and structure are similar to the mineral phase in bone.

HA, TCP, HA/TCP, CaCO3, Geopolymers,……

This presentation is focused on: pure and doped bioactive glass.



Slide 6

C1

Les biomatériaux de comblement offrent une alternative intéressante aux greffes osseuses de part leur grande disponibilité et par l'absence de risque de transmission de virus. Parmi les biomatériaux synthétiques existant notre choix s'est porté sur les verres bioactifs. Ces matériaux présentent des propriétés intéressantes comme la biocompatibilité, sur laquelle je reviendrai par la suite, ou encore la bioactivité qui s'explique par une suite de réactions physico-chimiques à l'interface verre-milieu.
Ces réactions consistent en trois grandes phases : tout d'abord, la dissolution du réseau de silice du verre, suivie par la formation d'un gel vitreus de silice riche en SiO2 et enfin la formation d'une couche d'hydroxyapatite en surface.
En fait l'hydroxyapatite correspond à la phase minérale majoritaire de l'os et elle permet une meilleure accroche de l'os à l'implant.

Notre premier axe d'étude a été de comprendre l'influence de l'introduction d'élements à l'état de trace sur la bioactivité. Les effets de 3 éléments ont été étudiés : le Mg, le Zn et le Sr. L'introduction de ces éléments permet de controler la cinétique de formation de la couche d'apatite, afin de disposer de matériaux dont on peut controler la cinétique de bioactivité. En effet, certaines pathologies nécessitent une repousse osseuse plus ou moins rapide.

CRI, 5/3/2007

Chemical synthesis of bioactive glass: 46S6

weight %	SiO_2	CaO	Na_2O	P_2O_5
46S6	**46**	24	24	**6**

Melt-dervide method

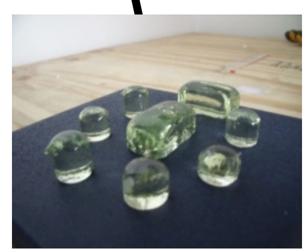
* Preparation of powder - Mixture
* Melting at 1300°C in Pt crucible with decarbonation step at 900°C
* Pouring into brass molds preheated at 500°C
* Annealing for 4h at T > Tg
* Grinding to obtain a grains less than 40 µm

Initial products-Mixing:
$CaSiO_3$, Na_2SiO_3 et $NaPO_3$

900°C, 1h
Calcinatation

1300°C, 3h
Melting

T ≈ Tg (552°), 4h
Annealing



- *Bioactive Glass: 46S6 as reference*
- *Bioactive glass doped with Zinc as trace element:*

  Zn-46S6: wt % of Zn: 0.02 to 0.1

"In vitro" assays

	Ionic concentrations 10^{-3} mol.L^{-1}						
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	2.5	1.5	148.8	4.2	1.0
Blood plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0

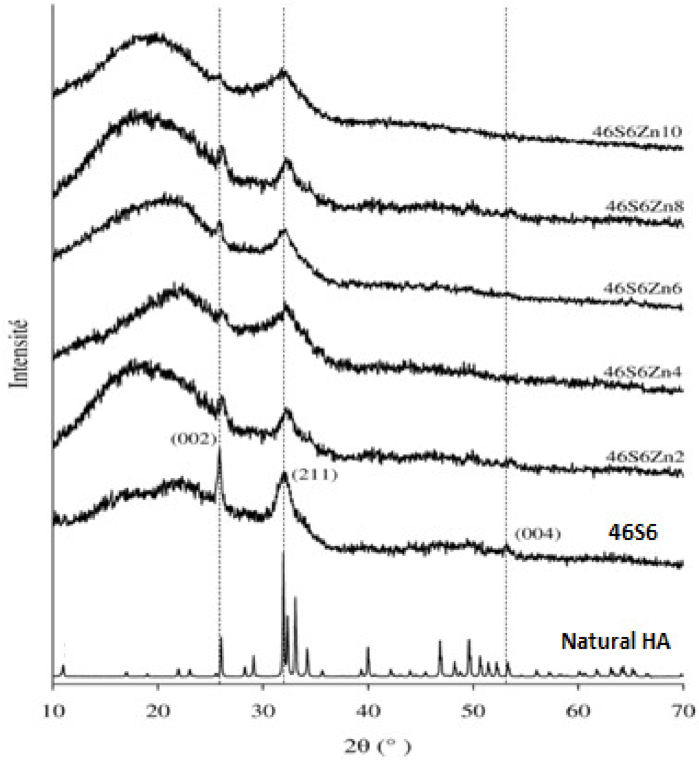
- SBF synthesis (Simulated Body Fluid)
- Compounds soaked in SBF
- Soaking periods: 1, 3, 7, 15 and 30 days.
- Compounds maintained at 37° under controlled agitation (50 tours/min)

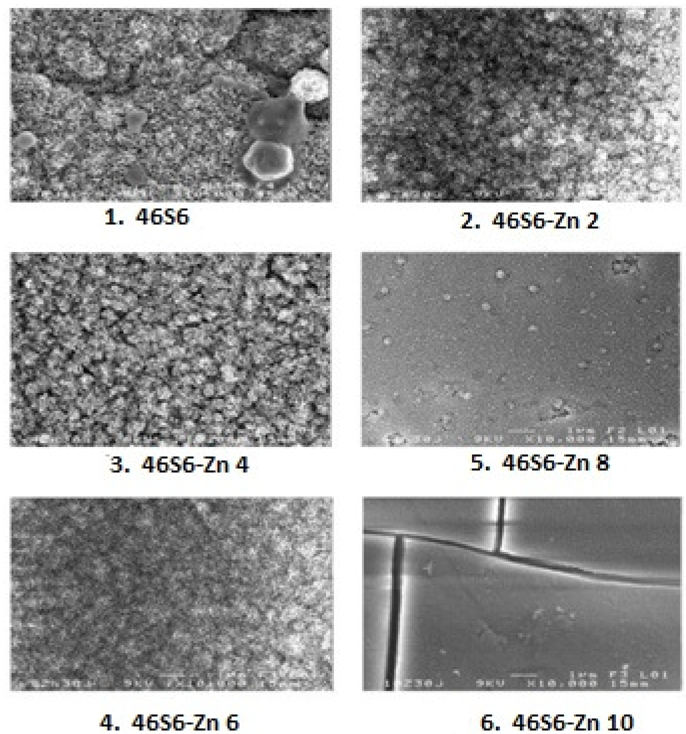
*Pysicochemical studies: XRD, SEM, MAS-NMR and ICP-OES

*Biological evaluations: in vivo assays Oxidative balance

Incubator

Zn effect on the apatite formation





XRD and SEM 30 days of soaking in SBF

*Crystallisation quality of the calcium phosphate, formed at the surface of glass, decreases with the increase of Zn amount.

* This tendency is confirmed by the graphs obtained by SEM.

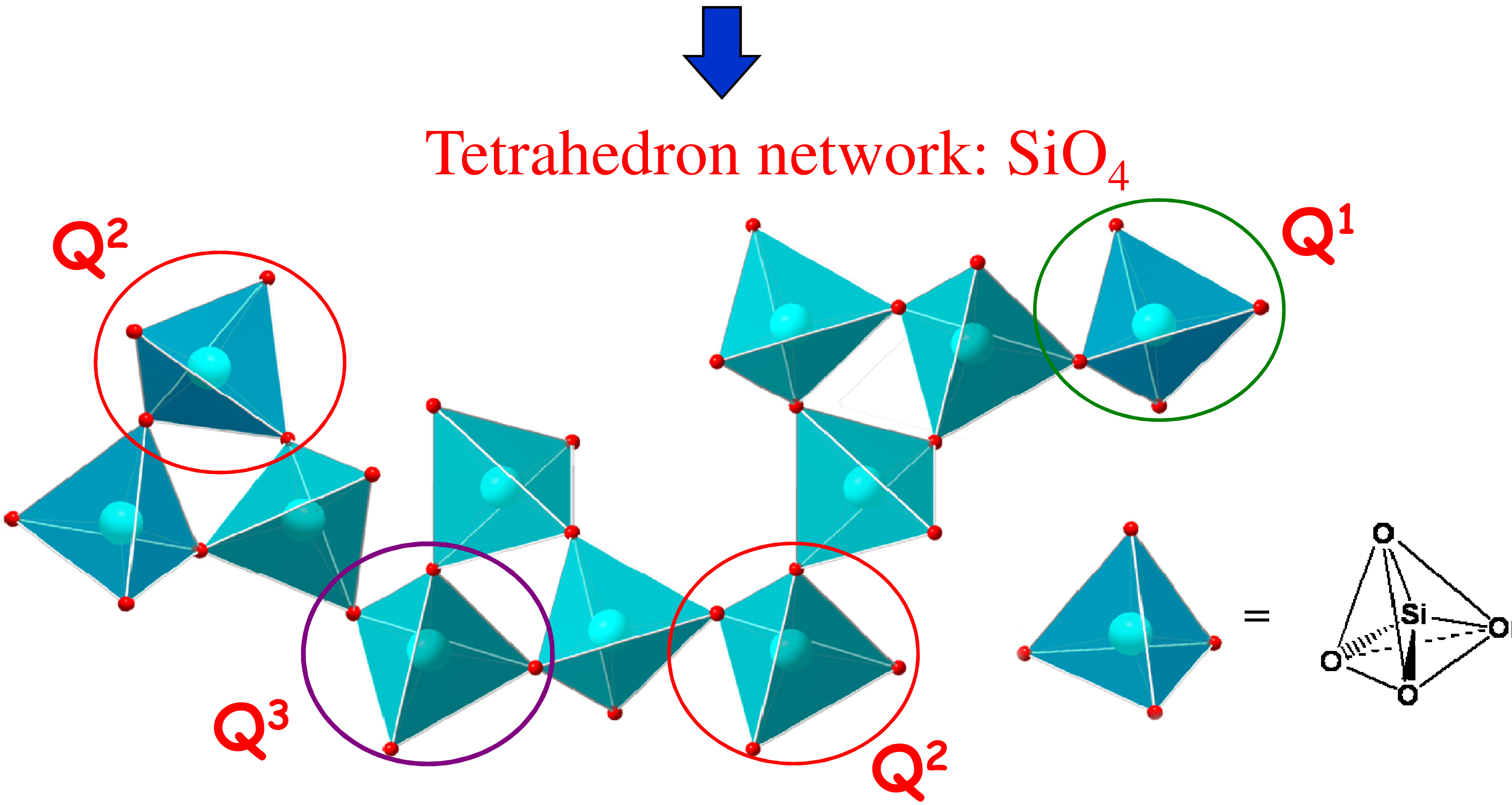
The presence of Zinc affect the HA properties and slows the its formation.

Advantages: *Desirable when the activity of bone metabolism is low.
*Suitables for the elderly

MAS-NMR: Mechanism of the Glass dissolution and Calcium Phosphate formation

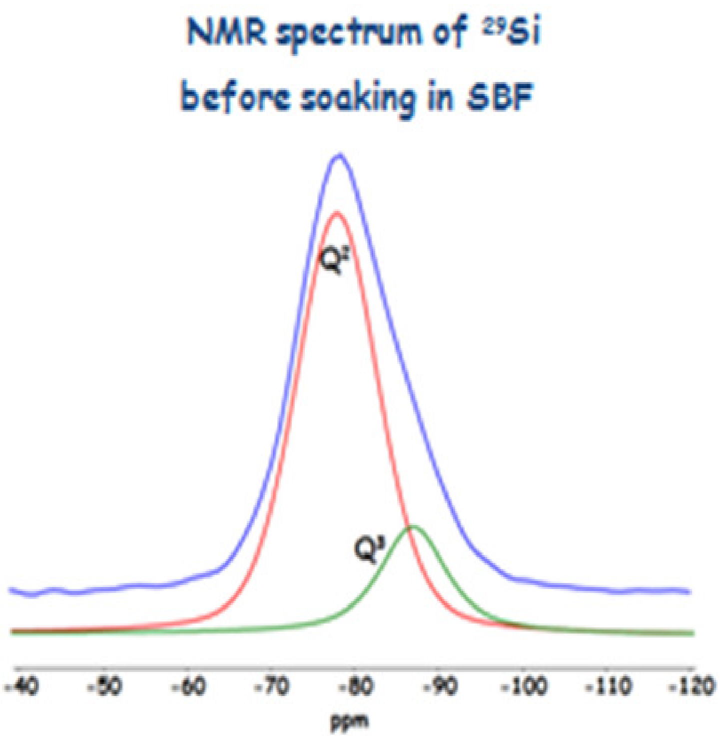
- Structural model of glass of silica:

Tetrahedron network: SiO_4



Q^n, n: number of bridging oxygens

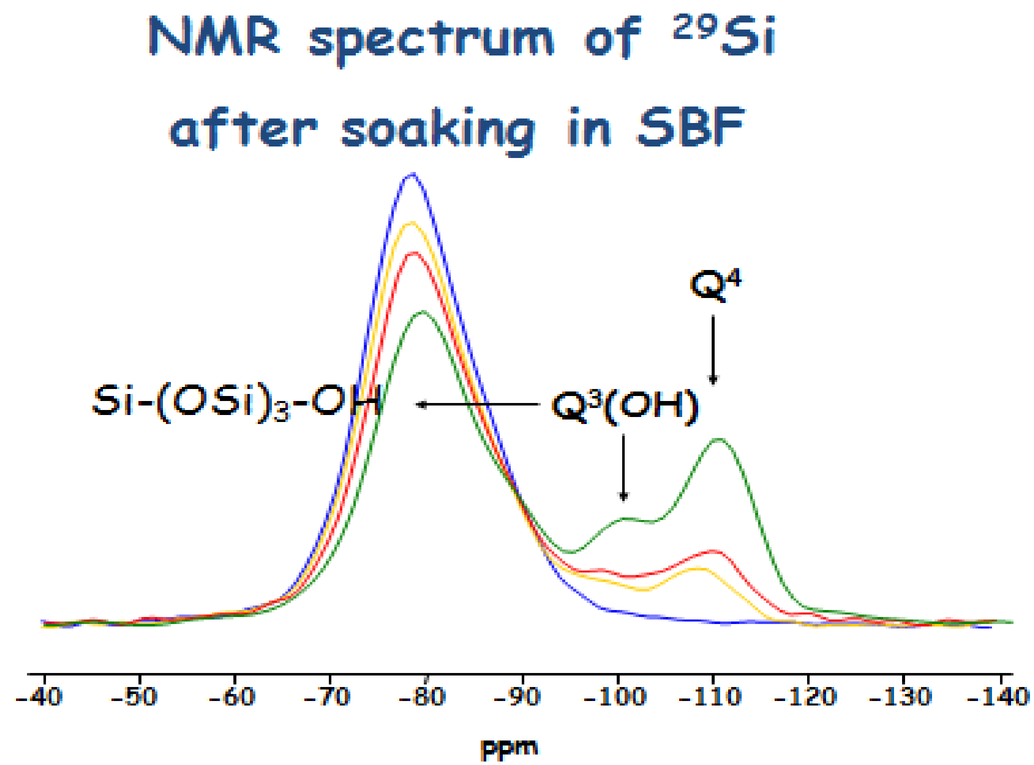
MAS-NMR: Bioactive Glass dissolution after « in vitro » assays



Bioactive Glass 46S6

: before immersion
: Q^2 $\delta \approx -78$ ppm
: Q^3 $\delta \approx -87$ ppm

The 46S6 structure is composed with:
80% of Q^2 and 20% of Q^3 species

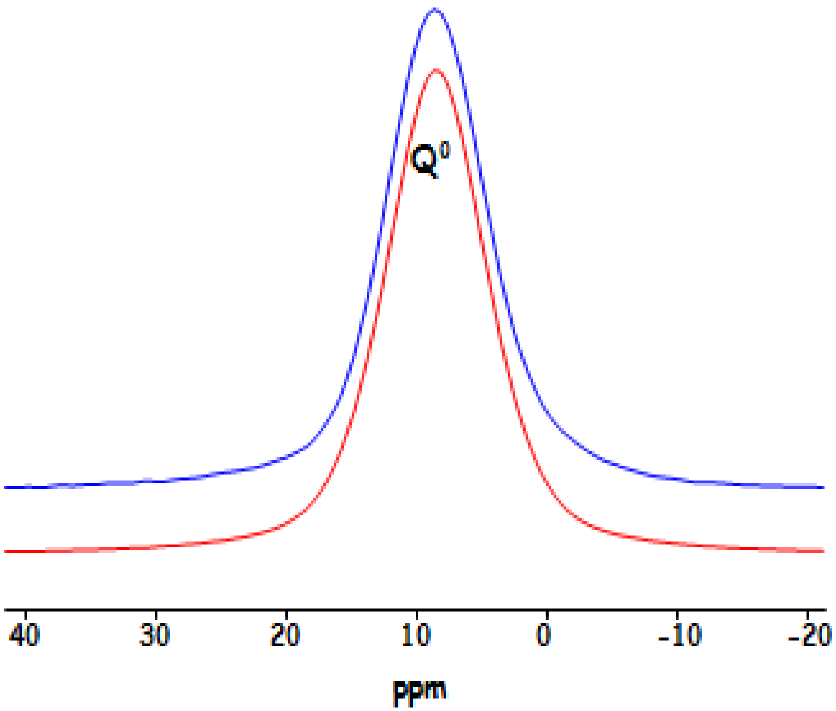


:Before soaking
:1 day of soaking
: 7 days of soaking
:15 days of soaking

Appearance of two new species:
$Q^3(OH)$ and Q^4

MAS-NMR: Calcium Phosphate formation after « in vitro » assays

NMR spectrum of ^{31}P
before soaking in SBF



NMR spectrum of ^{31}P
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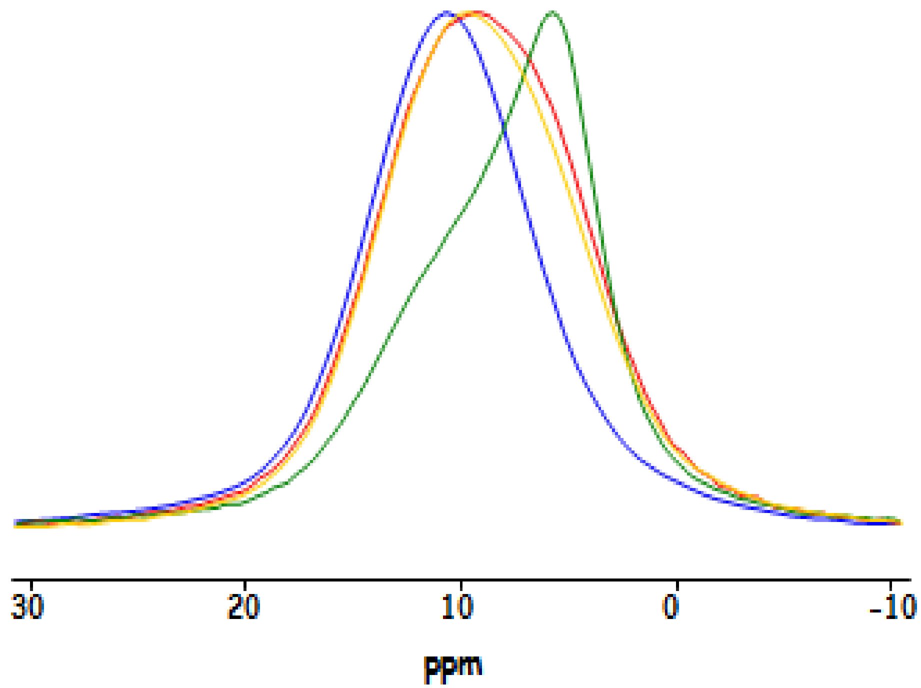


: Before soaking

: δ= 9 ppm

Phosphorus:
in Orthophosphate environment

:Before soaking

:1 day of soaking

: 7 days of soaking

:15 days of soaking, δ= 9 ppm

Appearance of new specy after soaking

Bioactive glasses

Quaternary system: SiO_2 – Na_2O – CaO – P_2O_5

↓

Bioactivity: *Interactions Glasses- SBF liquide
*Chemical reactivty and bioactivity depending on the glass chemical composition
*Hydroxyapatite layer (HA),

$Ca_{10}(PO_4)_6(OH)_2$: after soaking in SBF liquide

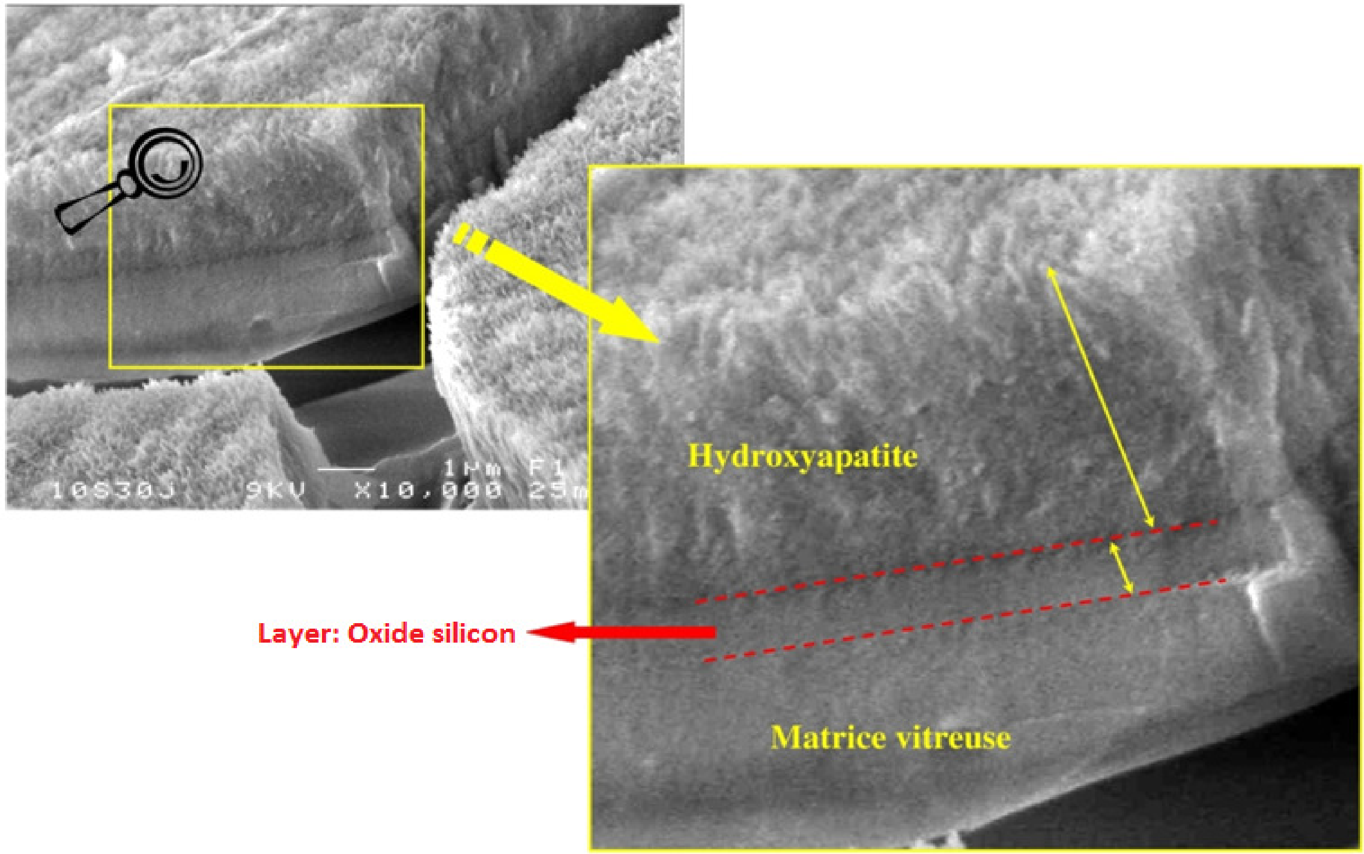
↓

Hydroxyapatite similar to mineral bone part

↓

Good bone bonding

C11



Surface of bioactive glass
30 days after soaking in SBF

1. Dissolution of the vitreous matrix
2. Vitreous gel formation
3. Precipitation of the hydroxyapatite layer

Slide 14

C11 Les biomatériaux de comblement offrent une alternative intéressante aux greffes osseuses de part leur grande disponibilité et par l'absence de risque de transmission de virus. Parmi les biomatériaux synthétiques existant notre choix s'est porté sur les verres bioactifs. Ces matériaux présentent des propriétés intéressantes comme la biocompatibilité, sur laquelle je reviendrai par la suite, ou encore la bioactivité qui s'explique par une suite de réactions physico-chimiques à l'interface verre-milieu.
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CRI, 5/3/2007

Comparison of the kinetic of bioactivity of: 46S6 and Zn-46S6

- The Inductively Coupled Plasma- Optical Emission Spectroscopy: (ICP-OES) was employed:

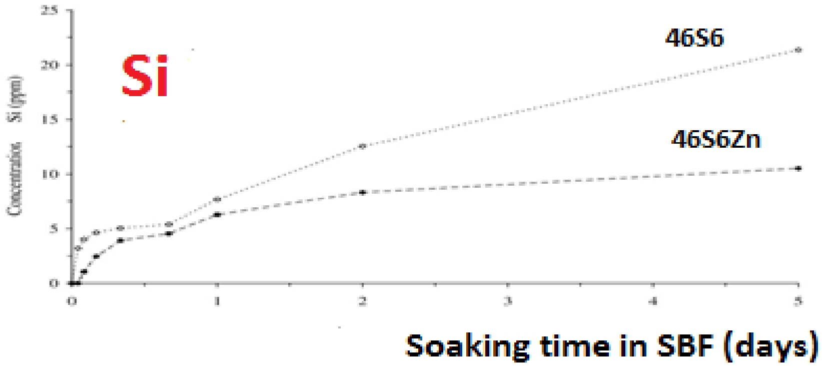
  It highlights the ionic exchanges between compounds and SBF solution after soaking vesus time.

- The ICP-OES analysis were carried out on the SBF solution after each time of soaking of bioactive glasse

- Sensitivity less than 1µg/g

- High accuracy

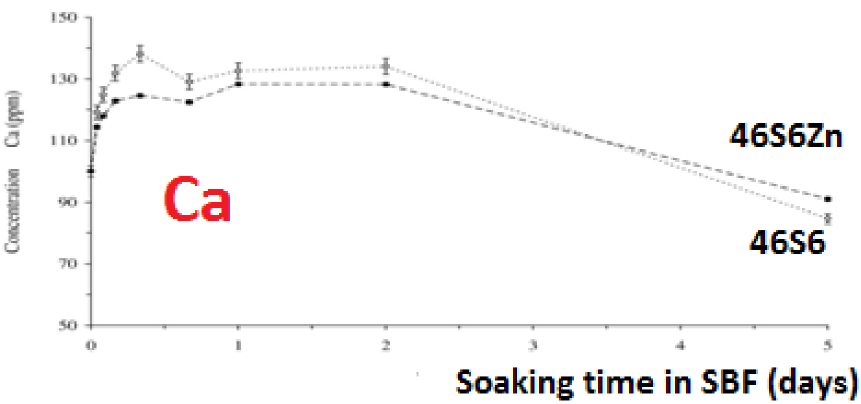
Evolutions of Si, Ca and P concentrations with the soaking time in SBF



variation of **Si** concentration in the SBF:
different in pure glass 46S6 than that in Zn-46S6.

-5 days after soaking:
Ţ(Si, 46S6) > Ţ(Si, Zn-46S6) in SBF

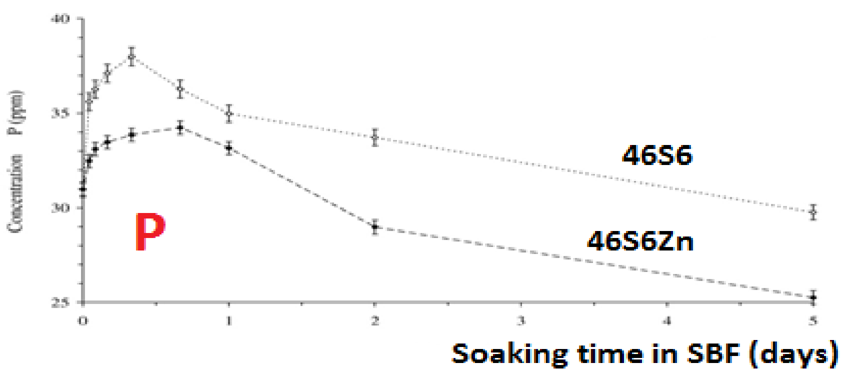
The presence of Zn slows the glass dissolution.



* **Variation of Ca** concentrations in SBF:
- reached a maximum after 12 hours of soaking,
- stabilization between 12 hours and 2 days

- 5 days after soaking:
Ţ(Ca, 46S6) ~ Ţ(Ca, Zn-46S6) in SBF

Variation of Ca: is similar in 46S6 and Zn-46S6.



Variation of **P** concentration differe between 46S6 and Zn-46S6.
Important relaese of P after 12 hours of soaking

- 5 days after soaking:
Ţ(P, 46S6) > Ţ(P, Zn-46S6) in SBF

The presence of Zn: *slows the dissolution of glass surface
* delays the Calcium Phospahte formation

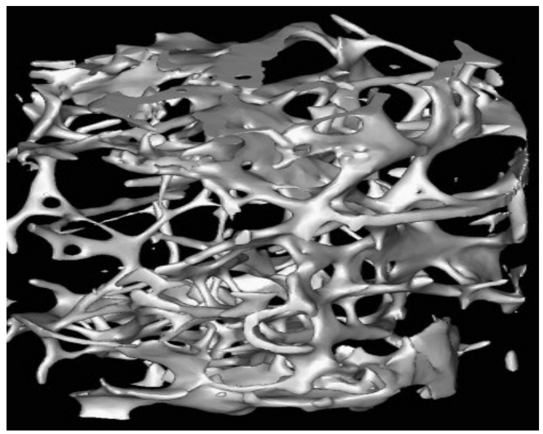
In Vivo Experiment :Implantation of Zn-46S6 in bone defect: Effects on the oxidative balance

Irradiation of bone → Osteoporosis → destroyed the oxidative balance

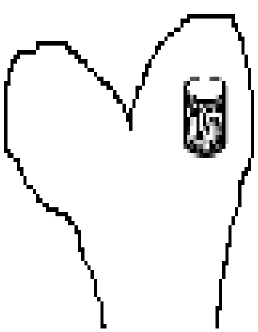
Anesthesia of the rats
(xylazine+ ketamine)

Irradiation of rats by Gamma ray:
^{60}Co (1,2,4 and 15 Gy)

3Weeks after irradiation:
appearance of the biological effect of irradiation:
ostéoporosis, disconnection of trabeculae



Osteoporosis destroyed the oxidative balance:
*increased the oxidant enzym (MDA)
*decreased the antioxidant enzym(SOD, CAT GPx)



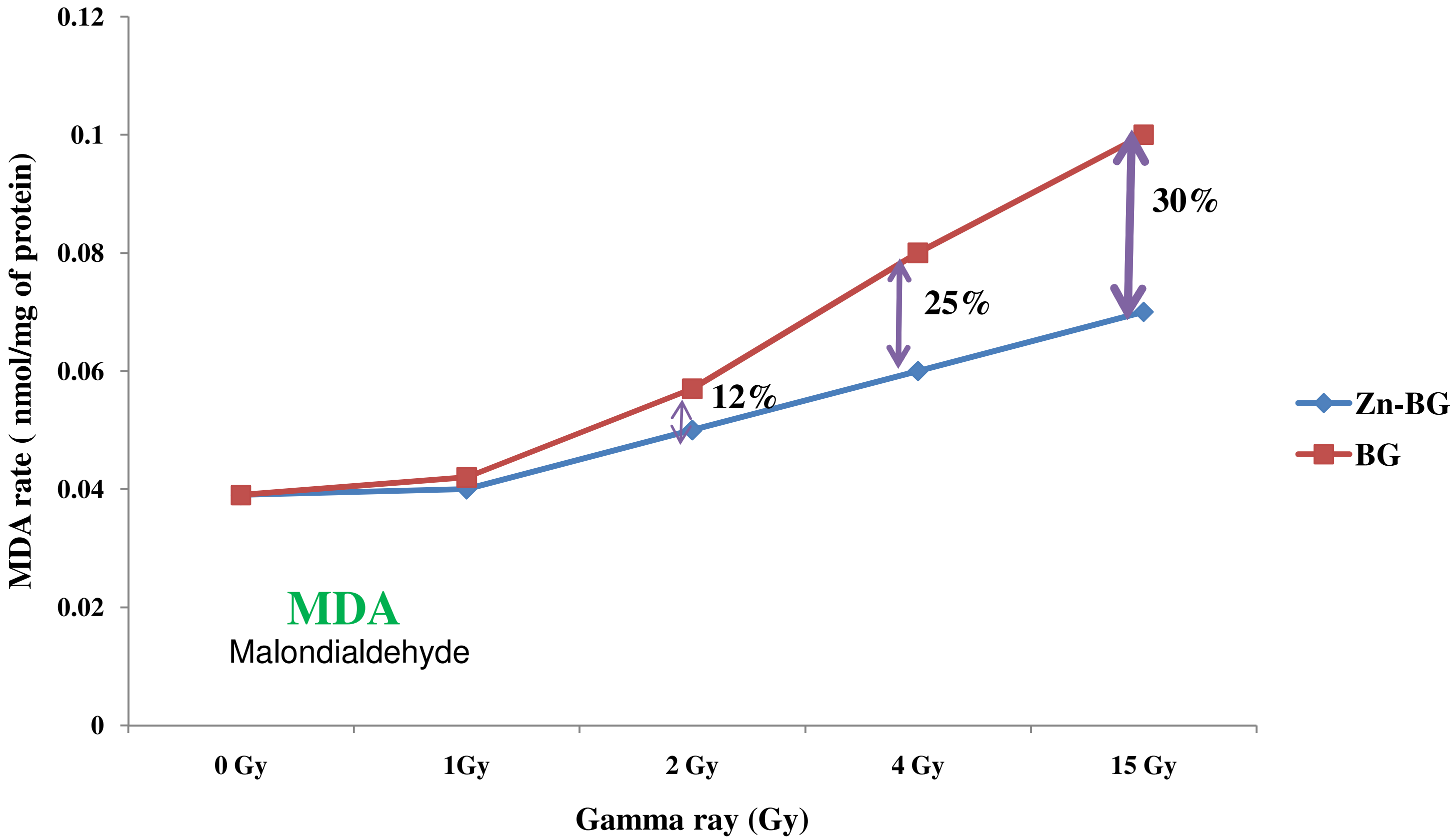
Preparation of the biomaterial to be implanted
3weeks after irradiation

- 3-mm diameter, 4-mm length
- 62% porosity

sterilizing the biomaterial by
the Gamma ray ^{60}Co

4 Weeks after implantation, tissue harvest
femur implanted with biomaterial

Effect of Zn-BG implantation on MDA (oxidant enzym) in bone

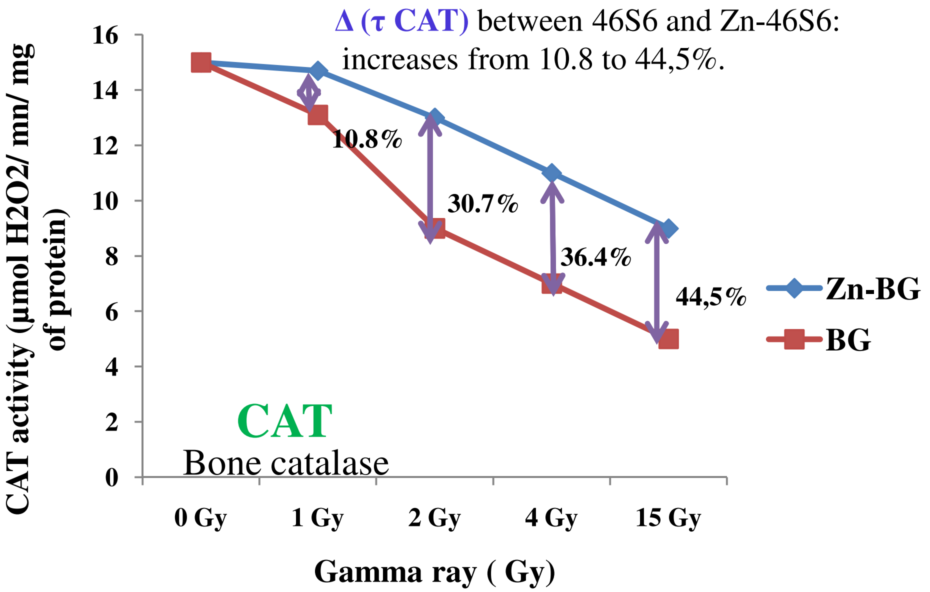


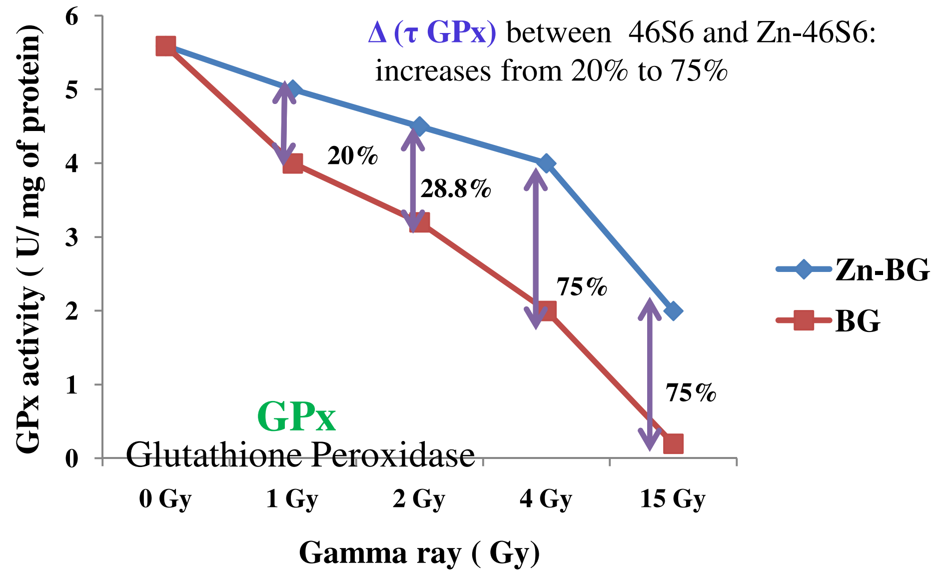
Bone malondialdehyde (MDA) after implantation with 46S6 and Zn-46S6 in rats irradiated with gamma ray

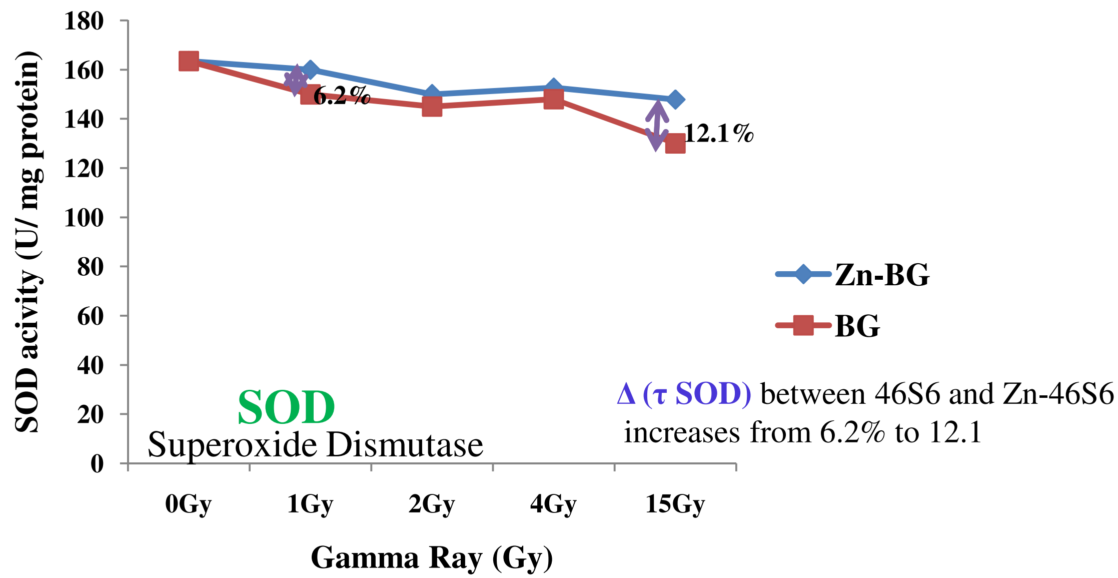
- After irradiation of rats, we show an increase of MDA in all groups compared to the control.
- Δ (τ MDA) between 46S6 and Zn-46S6 increases from 12% to 30%

The presence of Zn reduce the increase of oxidant enzym: MDA

Effect of Zn-BG implantation on CAT, SOD and GPx (antioxidant enzyms)







(CAT), (GPx) and (SOD), after implantation with BG (46S6) and Zn-BG (Zn-46S6) in irradiated rats.

The presence of Zn reduce the decrease of antioxidant enzyms: CAT, GPx and SOD

Conclusions:

- The presence of Zn: *slows the dissolution of glass surface
  * delays the Calcium Phospahte formation

- Zinc has an important role in regulating the balance (oxidant / antioxidant).
  * It reduces the increase of oxidant enzym: MDA.
  * It reduces the decrease of antioxidant enzyms:CAT, GPx and SO

- Zn doped glass present an interest, it can be adapted as biomaterials when the bone metabolism acitivity is low.

Biomaterials needs

- 25 billion of euro
  annual growth rate of 5 to 7%

- A third of this market is explored in Europe

- Biomaterials in the bone site:
  8 billion of euro with annual growth rate of 7%

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