BioMg®250  Bioabsorbable Implant Alloy

A. Alloy Design
A.1. Alloying for mechanical properties

Mechanical properties double those of bioabsorbable polymers (only 110 MPa tensile strength) are required for structural bone fixation; both for surgical insertion and also to insure rigid fixation of the repaired bone array. Such > 200 MPa strength levels are seen in Mg alloys that are hardened by Al; but that element is suspect in dementia, Alzheimer’s disease and bone dissolution. Therefore an alternate strengthening mechanism is demanded for bioabsorbable Mg alloys.

Therefore, nanoMAG selected a new combination of alloying elements to fortify the mechanical properties of the Mg base. Narrowing the search to elements that are established nutrients to the body, nanoMAG utilized Quantum Mechanics First Principles to select and optimize ternary additions of odd-sized elements; both positive and negative sizes to the Mg atom and to each other. At the same time, the principles of micro alloying were practiced – to capture synergisms amongst the alloying elements at low levels while avoiding the detrimental effects on corrosion and ductility of phases introduced by excessive alloying.

Thus the selected ternary alloying elements were Zn, Ca and Mn, based on the +/- misfits and strengthening potencies seen in Table I. Significant +/- oddness to Mg was achieved at -38 to +28% in size; with 66% oddness between Ca and Zn. The mixing enthalpy between Ca and Zn is negatively large at -22kJ/mol, an order of magnitude larger than Mg-Ca. Thus there is a strong attractive interaction between Ca and Zn in the Mg matrix. Also, the pairing of large and small solute elements lowers misfit of the new phase with the matrix, enabling easier nucleation. The free energy of the alloy system can then be lowered during processing by the segregation of alloying elements – to dislocations, grain boundaries and to nucleate nano-sized phases.

Table I. Alloying Elements in BioMg®250 and Misfits from Mg atom

<table>
<thead>
<tr>
<th>Alloying Element in Mg Base</th>
<th>% Size Misfit from Mg Atom</th>
<th>Chemical Misfit from Mg Atom</th>
<th>Strengthening Potency, MPa/Atomic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>-15</td>
<td>+0.4</td>
<td>33</td>
</tr>
<tr>
<td>Ca</td>
<td>+28</td>
<td>-1.4</td>
<td>84</td>
</tr>
<tr>
<td>Mn</td>
<td>-38</td>
<td>+2.1</td>
<td>121</td>
</tr>
</tbody>
</table>

Indeed, that is the case wherein clusters or short range ordered zones (known as Guinier-Preston (GP) zones) form on the basal {0001} planes of Mg. These GP zones are one atomic layer thick (< 0.5 nm) and about 15 nm in diameter (see Figure 1, Allison and Makiheni). The array of Zn and Ca atoms in these ordered zones is pictured in Figure 2. The Zn to Ca ratio is 2:1
and intercluster distance is 10 nm on the basal plane (Marquis and Purvis). The population is about $10^{22}$ to $10^{23}/m^3$. These zones resist dislocation deformation on the basal planes, shunting this deformation to prismatic planes for higher strength and formability. The end result is tensile strengths of 250 – 320 MPa and elongations of 12 - 20 %, depending on the process steps (see Tables II, III). The excellent ductility and formability are demonstrated in Figure 4.

Figure 1. Electron micrograph (STEM) images of GP zone in BioMg 250, a) brightfield image, b) darkfield image in which bright atoms are Zn and Ca. (Allison, Makiheni)

Figure 2. Array of Zn and Ca atoms in GP zone on basal plane of Mg matrix of BioMg 250.
In addition to its role in solid solution hardening, Mn additions inserted spherical nm size α Mn particles that refine the grain size to amplify the Hall-Petch strengthening (see Figure 3).

![100 nm](image)

Figure 3. Spherical α Mn particles, 10 – 120 nm diameter, for grain refinement (Allison, Makiheni)

<table>
<thead>
<tr>
<th>Table II. Mechanical Properties of BioMg 250 Plates and Screws</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Process</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>A for high strength</td>
</tr>
<tr>
<td>B for medium strength</td>
</tr>
<tr>
<td>C for highest strength</td>
</tr>
</tbody>
</table>

Table III. Mechanical Properties of BioMg 250 Wire

a. 1.1 mm Diameter

<table>
<thead>
<tr>
<th>Condition</th>
<th>YS, MPa</th>
<th>UTS, MPa</th>
<th>Elongation, %</th>
<th>Bendability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annealed</td>
<td>230</td>
<td>270</td>
<td>19</td>
<td>1.6 mm radius</td>
</tr>
<tr>
<td>18% Cold Work</td>
<td>360</td>
<td>360</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>34% Cold Work</td>
<td>380</td>
<td>380</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>49% Cold Work</td>
<td>400</td>
<td>400</td>
<td>3</td>
<td>2.4 mm radius</td>
</tr>
</tbody>
</table>

b. 0.3 mm Diameter

<table>
<thead>
<tr>
<th>Condition</th>
<th>YS, MPa</th>
<th>UTS, MPa</th>
<th>Elongation, %</th>
<th>Bendability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Work 50%</td>
<td>400</td>
<td>400</td>
<td>3</td>
<td>&lt; 1.2 mm radius</td>
</tr>
</tbody>
</table>
A.2. **Alloying for biocorrosion.** Small alloying additions of Zn, Ca and Mn were all beneficial to corrosion resistance in Synthetic Body Fluids (SBF) at 37°C. However, with each element, excessive additions introduced extra phases that led to excessive degradation rate. Thus microalloying with the three elements resulted in a rate intermediate between ZK60 (too fast) and AZ91D (too slow) for bioabsorption in 1 year (Figure 5).

A.3. **Alloying for biocompatibility**

A.3.1. The base **Magnesium (Mg)** is an osteoconductive, biocompatible and degradable material. Magnesium is an essential element in the body. Mg$^{2+}$ is the fourth most abundant cation in the human body and is a co-factor to many enzymes and metabolic processes (1-5). Yoshizawa and Sfeir, et al, (6) identified extracellular matrix proteins and transcription factors positively affected by Mg$^{2+}$ that are responsible for the enhanced bone generation observed around degradable Mg orthopaedic/craniofacial devices. Cheng (7) found that Mg interference screws for ACL fixation *in vivo* promoted the expression of bone morphogenic protein -2 (BMP-2) and vascular endothelial growth factor (VEGF) and fibrocartilaginous enthesis regeneration. *In vitro* and *in vivo* studies indicated that Mg-based implants have good biocompatibility with no chronic inflammatory reactions (8,9). Mg alloys better match bone strength and elastic modulus than Ti implants, thus mitigating stress shielding tendencies. Push-out testing revealed significantly greater pull-out force, ultimate shear strength and energy absorption in Mg implants than in Ti implants – with significantly higher bone-implant contact and bone volume with the Mg implants (4). Upon absorption, the volume surrounding the Mg implant becomes alkaline as an advantage.
over the harmful acidity around certain polymer implants. The rapidly growing body of evidence for magnesium’s osteogenic enhancing effects suggests it could be harnessed to improve bone defect regeneration. In a 48 patient clinical study, Dewei-Zhao (10) found that biodegradable Mg screws provided successful fixation of vascularized bone graft in treating osteonecrosis of the femoral head (ONFH).

**References on Mg:**

**A.3.2. Calcium (Ca)** is known to be the essential nutrient element and the most abundant mineral in the body, about 1000 - 1300 g for a healthy adult. It can regulate the normal physiological function of the organs, tissues and systems. Ca is the major mineral component of bone and teeth, playing a crucial role in the formation of bone. Ca can also promote the activity of enzymes and the activity of many enzymes involved in cell metabolism requires the activation of Ca ion, such as lipase and amylase (1).

**Reference on Ca:**
A.3.3. **Zinc (Zn)** is a critical alloying addition to BioMg 250. Its benefits are many, as follows:

**Corrosion** - Reducing corrosion and H₂ release

1. The oxidized Zn in Mg(OH)₂ from the replacement of the Mg²⁺ cations suppresses the penetration of the Cl⁻ anions into Mg(OH)₂ providing a good barrier that improves the corrosion resistance of the quaternary alloy.
2. Zn is present at 1.0 – 1.7 % in the corrosion layer in 52 week *in vivo* tests of BioMg 250 (nanoMAG data).
3. Zn forms Mg₂Zn particles that are anodic to the α Mg matrix and Ca₃Mg₆Zn₂ particles that are cathodic – thus allowing control of corrosion

**Grain Size** – Finer grains

1. Zn decreases the grain size, as cast and as thermomechanically processed (TMP)
2. Mg₂Zn and Ca₃Mg₆Zn₂ restrain grain growth during annealing

**Strength** – Higher strength with ductility

1. By reducing grain size, strength is increased in proportion to grain size⁻¹/² (Hall-Petch strengthening). Sub- grain size can be reduced to 100 nanometers.
2. The Zn atom has odd atomic size (-15 %) and odd chemical misfit (+0.4) compared to the matrix Mg atom
3. This oddness induces solid solution strengthening
4. This oddness strengthens by a) clustering in the α matrix, b) short-range ordered nanometer zones of 0.5 by 15 nm, and c) segregation to sub-grain and grain boundaries – all enhanced by the co-presence of Ca atoms (Orowan Strengthening )

**Bone Growth** – Enhanced

1. Zn enhances bone formation with mineralization and is an essential element of osteoblastic proliferation (Kallyanashis)
2. Zn has been implicated in the prevention and reversal of the osteoporosis process. (Fielding)
3. Zn⁺² has anti-inflammatory/antibacterial effect (Moreno-Eutimio)
4. Zn was found at 0.3 % in new bone grown *in vivo* on BioMg 250 in 52 weeks (nanoMAG data)

**Bone Growth Mechanisms** –

1. Zn has a significant role in the metabolism of proteins, carbohydrates and lipids (Molokwu)
2. Zn release during skeletal breakdown can inhibit osteoclastic bone resorption (Fielding)
3. The antibacterial effect of Zn⁺² induces faster bone healing by preventing bacterial colonization and degradation of premature membrane (Chou)
4. Zn stimulates cellular proliferation and differentiation of osteoblastic cells and inhibits the activity and differentiation of osteoclastic cells (Yang)
5. Zn\(^{+2}\) increases ATPase activity and regulates transcription of osteoblastic differentiation genes such as collagen, osteopontin and osteocalcin (Ghorbani)

References on Zn:

A.3.4. Manganese (Mn) is a critical alloying addition to BioMg 250. Its benefits are manifold, as follows:

**Corrosion** - Reducing corrosion and H\(_2\) release
1. Mn reduces the cathodic current density and H\(_2\) release from Mg-Zn-Ca alloys [Zakiyuddin]
2. “The oxidized Mn in Mg(OH)\(_2\) from the replacement of the Mg\(^{2+}\) cations suppresses the penetration of the Cl\(^-\) anions into Mg(OH)\(_2\) providing a good barrier that improves the corrosion resistance of the quaternary alloy.” [Zakiyuddin]
3. First-principles analysis show that Mn4+ in MgO increases the resistance of the oxide film towards water activated degradation [Kwak]
4. Mn in BioMg 250 reduced corrosion and H\(_2\) release by 40% [in vitro SBF tests at nanoMAG ]

**Grain size** – Mn reduces the as-cast and processed grain size [nanoMAG data, Tong]

**Strengthening** –
1. Mn increases the yield and tensile strengths of the Mg-Zn-Ca base [nanoMAG data, Tong]
2. Mn is strong solid solution hardener of Mg due to size misfit and chemical misfit of atoms (Yasi)

**Bone Growth** – Mn enhances
1. Mn essential for growth and development of bones [Liu, Bellof], vital to bone formation [Liu, Bae]
2. Mn increased Bone Mineral Density(BMD) in lumbar vertibra and in femur [Bae]

**Bone Growth Mechanisms of Mn**
1. Mn is beneficial to osteoblastic cell differentiation, biocompatibility and bone metabolism [Liu, Sima, Bigi, Paluszkiewicz]
2. Mn in calcium phosphate can improve production of osteocalcin in osteoblasts, even more effectively than Mg [Liu, Bracci]
3. Mn has important role in carbohydrate metabolism and the synthesis of mucopolysaccharides within the bone.[Liu, Bae]
4. Mn can promote bone-related marker gene expression in osteoblastic cells [Liu]
5. Mn deficiency delays osteogenesis and reduces osteoblast activity [Liu, Li]
6. Mn is present at 0.1-8 mg/kg (0.01-0.8 %) in bone and is essential for glycosal tranferase [Bellof, Aaseth]
7. Mn is present at 0.11 % in new bone formed in vivo on BioMg 250 (nanoMAG data)
8. Mn$^{2+}$, with an ionic radius similar to that of Ca$^{2+}$, is known to enter cells through the voltage-gated Ca channels [Chesnick]
9. Mn in calcium phosphate is not cytotoxic, with excellent bioactivity and spread of cells over the surface [Sima]
10. Mn$^{2+}$ increases the liquid-bonding affinity of integrins, a large family of receptors responsible for activation of cell adherence processes [Paluszkiewicz, Bracci]

References on Mn::

B. Alloying effects in vivo
The reaction of BioMg 250 implants and its alloying elements was measured during 52 week in vivo tests in rabbits at North Carolina State A &T. By Scanning Electron Microscope (SEM) images and linear scans, the alloying elements were located in a) the residual implant, b) a transition corrosion layer and c) in new bone that encapsulated and replaced the implant. The SEM images for Mg, Zn, Ca and Mn are seen in Figures 6 - 12 , along with O and P. Quantitative measurements of alloying contents in the transition corrosion layer and new bone are included in Table IV.
Figure 6. SEM of residual implant after 52 weeks in vivo, with corrosion reaction layer and encapsulating new bone.

Figure 7. Mg Map

Figure 8. Ca Map

Figure 9. P Map
Figure 10. Zn Map

Figure 11. Mn

Figure 12. O
Table IV. SEM linear scan results on BioMg 250 implant after 52 weeks *in vivo* in rabbit.

<table>
<thead>
<tr>
<th>Element</th>
<th>% in Transition Layer</th>
<th>% in New Encapsulating Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>23 - 30</td>
<td>1.4 - 6.6</td>
</tr>
<tr>
<td>Zn</td>
<td>1.0 - 1.7</td>
<td>0.22 - 0.31</td>
</tr>
<tr>
<td>Ca</td>
<td>11 - 15</td>
<td>38 - 41</td>
</tr>
<tr>
<td>Mn</td>
<td>0.2 - 0.5</td>
<td>0.08 - 0.14</td>
</tr>
<tr>
<td>P</td>
<td>4 - 10</td>
<td>18 - 19</td>
</tr>
<tr>
<td>O</td>
<td>49 - 53</td>
<td>37 - 40</td>
</tr>
</tbody>
</table>

In concert with the literature and the role in corrosion, Zn and Mn were found in the transition corrosion layer; in the case of Zn segregated in particles (Figure 10). All the elements were found alloyed in the new bone, probably related to their osteoconductivity.

April 18, 2016