Bioactive coatings on Zinc-based alloys for sustainable absorbable orthopedic implants

Metallic materials are one of the four main classes of biomaterials, which are widely used in broad biomedical applications. This is due to the fact that many metal alloys exhibit suitable biocompatibility, mechanical properties and resistance to corrosion. Recently, **biodegradable or absorbable metals and alloys** have emerged and their development become one of the hottest research topics in biomaterials science. This new class of biomaterials is expected to be used for implants with a temporary function or to act as a temporary support in reparative and regenerative medicine. **These biodegradable/absorbable metals are more sustainable than permanent implants as they reduce the need of costly and risky additional surgeries for either implant replacement or removal.**
In this perspective, a good **sustainable absorbable metal implant** should combine **appropriate mechanical properties, biocompatibility and controlled degradation rate under physiological conditions**. Indeed, the rate of (bio)degradation should be fast enough to avoid toxic effects and slow enough to allow the regeneration of the native tissue.

Over the last two decades, Fe and Mg based alloys were extensively studied as potential candidates for absorbable metals in cardiovascular (vascular stents) and orthopedic (bone fixation devices) applications. This interest is mainly based on the observation that Fe and Mg are essential trace elements in the human body, as they are involved in many biochemical processes. Unfortunately, Mg alloys have a rapid corrosion rate in biological media provoking the formation of hydrogen gas pockets near the implant. Fe alloys have a slow corrosion rate and the corrosion products have a long retention time in the body which may cause medical complications.

In 2013, Zn metal and **Zn-based alloys** were introduced as **good candidates to build absorbable metallic implants** thanks to their moderate corrosion rate, biocompatibility and acceptable mechanical properties. Zn-based alloys have been the subject of a vast literature, in the context of biomedical applications, particularly regarding their microstructures and the resulting mechanical properties (1, 2). However, significant improvements are needed regarding their **surface behavior** in biological media. The main issue lies on tuning their biodegradation rates: in one hand, a slow rate leads to a long-term retention of the implant after accomplishing its function; on the other hand, a significant release of Zn^{2+} ions during the biodegradation process may induce cytotoxicity effect and influence other biological responses, such as osseointegration. Accordingly, improving the implant performance requires a control of interfacial processes involved in the biological media, including protein adsorption, thrombosis, biofilm formation, biodegradation, etc.

Recent works have suggested that improving the performance of Zn-based alloys in orthopedic applications requires particularly the acceleration of the degradation rate. We propose in this project a straightforward yet powerful strategy to tune the rate of degradation of these materials, while ensuring relevant biologically-relevant properties. For this purpose, we combine **electrochemical methods** with **mineralization processes** to build-up **bioactive porous coatings** made of calcium phosphate (CaP) and incorporating metal ions on **Zn-based biodegradable alloys**.

In this collaborative project between the teams “Caractérisation et Modélisation personnalisée du Système MUsculo-SqueleTtique” (C2MUST) and “Cellules Biomatériaux Bioréacteurs” (CBB) team of the BMBI lab (UTC) and “Laboratoire de Réactivité de Surface (LRS, Sorbonne Université), we aim to develop an innovative strategy for the design of bioactive coatings on Zn-based alloys, and evaluate the obtained materials in terms of biodegradation, cytotoxicity and microbial properties. The project includes 3 WPs that will be addressed by the two laboratories with their respective and complementary expertises.

WP1. Design of the bioactive coating

The surface functionalization of Zn-based biodegradable alloys is particularly difficult owing to the high reactivity of these materials in biological media (3). In this project, we intend to explore the growing

integration layer (GIL) method, recently developed by Huang and Yoshimura for titanium alloys (4), and combine it to mineralization to form a ceramic coating under electrochemical control at room temperature. Indeed, applying a high voltage to the electrode, yielding a current density in the range of 1 $A.cm^{-2}$, will induce an anodic dissolution of Zn into Zn^{2+} ions. The addition of calcium and phosphate ions in supersaturated solution will lead to the formation of a porous ceramic layer in which Zn^{2+} ions can be incorporated. The proposed method presents several advantages. First, it makes an integrated layer from bottom-to-top of the material, in a way similar to the well-known "conversion layer", thus, it adheres well on the substrate and prevents a major problem related to the formation of stress. Second, it is expected to exhibit a suitable biocompatibility due to the presence of calcium phosphate species. Third, it can modulate the biodegradation rate of the Zn alloy by means of galvanic corrosion between the coating and the substrate, as recently observed for coatings made with microarc oxidation method (4). Fourth, the addition of other ions, such as magnesium, selenium and strontium ions, may lead to their incorporation in the coating, thus providing a way to improve some biological properties (e.g. accelerating the healing, promoting cell differentiation, treating osteoporosis).
The obtained coatings will be fully characterized by means of surface science techniques available in LRS lab (including XPS, AFM, PM-IRRAS and Raman) and in BMBI lab (SEM, AFM, QCM-D).
The development of these coatings will be based on our expertise in (i) electrochemical methods and (ii) calcium phosphate mineralization processes.

WP2. Biodegradation assessment

The rate of biodegradation of the obtained coated Zn alloys will be of a primary importance for the reasons described above. These investigations will be conducted using a variety of electrochemical methods, including real-time monitoring of the corrosion potential (E_{corr}) and potentiodynamic polarization techniques. The experiments will be conducted in aqueous solutions simulating physiological conditions (type of ions, ionic strength, pH and temperature). Moreover, a biomimetic enzymatic system mimicking cellular activity will be used to evaluate the stability of the coating in the presence of oxidizing species (e.g. hydrogen peroxide) and organic acids (e.g. gluconic acid) produced by living cells.

WP3. Biological tests

Both microbial tests (supervised by Laëtitia Valentin, IE, LRS) and cell culture tests (supervised by Timothée Baudequin) will be conducted to provide a complete evaluation of the coatings developed in this project.
An inoculum for the development of biofilms on the coated Zn alloys will be prepared by culturing Staphylococcus aureus. The bacteriostatic efficacy will be evaluated by counting the number of bacterial colonies formed on the studied coated substrates. The total biomass and the metabolic activities of the biofilms will be also investigated using standards methods commonly used in the LRS microbiology lab. Moreover, the internal biofilm structure will be probed using confocal microscopy available in the SAPC platform (UTC).
Cell attachment, spreading, proliferation and viability will be evaluated thanks to metabolic activity testing (Alamar Blue, MTS), fluorescence microscopy (Live/Dead assay) and SEM observations. MC3T3-E1, a murine pre-osteoblastic cell line, will be used first. To evaluate further the impact of the biomaterials on osseointegration, the mesenchymal stem cell model C3H10T1/2 will be also used to evaluate the promotion of bone differentiation, starting with ALP staining and activity assays. Both direct (cells seeded on the material) and indirect (cells attached on plastic and material present in a culture insert) culture conditions will be evaluated. It will allow us to evaluate respectively the effect of the surface properties and the effect of the ion release on the cell behaviour.

References

(1) D. Hernández-Escobar et al. Acta Biomaterialia 97 (2019) 1-22.
(2) H. Kabir et al. Bioactive Materials 6 (2021) 836-879.
(3) W. Yuan et al. Bioactive Materials 7 (2022) 192-216.
(4) W. Yuan et al. ACS Biomater. Sci. Eng. 5 (2019) 487−497