Viability, Adhesion, and Morphology of Bone Marrow Stromal Cells on Four Magnesium-Zinc-Calcium Alloys

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Statement of Purpose: Magnesium (Mg) alloy-based implants hold great promise for orthopedic implant applications. Commercially available alloys (designed for industrial applications) contain possible toxic elements, which calls for development of new Mg alloys specifically designed for biomedical applications. From both biological and materials science points of view, Zinc (Zn) and Calcium (Ca) have been studied as promising alloying elements for Mg (Cipriano AF. Mater Sci Forum, THERMEC, 2013). The objective of this study was to investigate the cytocompatibility and degradation of four Mg-xZn-0.5Ca (x = 0.5, 1, 2, 4; weight %) alloys and their effects on adhesion and growth of bone marrow stromal cells (BMSCs) *in vitro*. BMSCs were used due to their important role of supplying progenitor cells needed for bone remodeling and repair (Rickard DJ. Dev Biol. 1994; 161: 218-28). BMSC adhesion and morphology on the Mg-xZn-0.5Ca alloys, pure Mg (PMg), and on the tissue culture plate around the samples were evaluated after 24 hr of incubation.

Methods: Rat BMSCs were harvested and cultured according to details found herein (Cipriano AF. J Biomed Nanotechnol, 2013; 9). BMSCs (P2) were seeded directly onto the sample surfaces at $4x10^4$ cells/cm^2 and incubated in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin under standard cell culture conditions for 24 hr. Samples used for fluorescence imaging were fixed with 4% formaldehyde and stained with 4',6-diamidino-2-phenylindole dilactate (DAPI) nucleic acid stain and Alexa Flour® 488 cytoskeleton stain. DAPI-stained nuclei were observed using a fluorescent microscope (Nikon Eclipse Ti; Nikon Instruments, Melville, NY) and counted per unit area to determine cell density. Samples used for scanning electron microscopy (Nova NanoSEM 450; FEI Co., Hillsboro, OR) and energy dispersive x-ray spectroscopy (AZtec Energy; Oxford Instruments, Oxfordshire, UK) were fixed in 3% glutaraldehyde, followed by serial dehydration in alcohol, critical point drying, and sputter coating. These samples were used to assess the degradation of the Mg-xZn-0.5Ca alloys and determine the cell-substrate interactions.

Results: The adhesion and morphology of the BMSCs was regulated by the degradation behavior of the Mg-xZn-0.5Ca alloys after 24 hr of cell culture (Figure 1a). BMSCs remained largely viable on and around the experimental samples and were within the same order of magnitude compared with the positive control (Figure 1b), indicating cytocompatibility of our Mg-xZn-0.5Ca alloys. The microstructure of each experimental sample had distinct features from one another (Figure 1c), which could be a factor influencing cell behavior.

Conclusions: The degradation of Mg-xZn-0.5Ca (x = 0.5, 1, 2, 4; weight %) alloys was shown to regulate BMSC viability, adhesion and growth after 24 hr of cell culture. Future work seeks to elucidate whether solubilized degradation products or surface degradation has a greater effect on modulating cellular activities. We also seek to investigate the cytocompatibility and degradation of our Mg-Zn-Ca alloys at 48-72 hr of incubation with BMSCs.

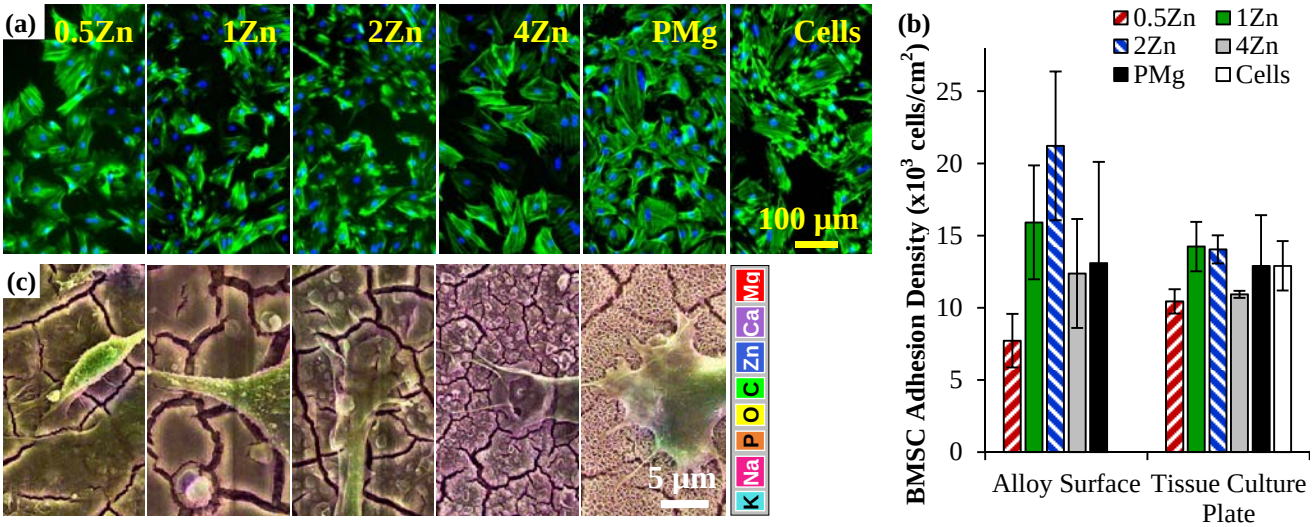

Figure 1: (a) Fluorescence images of BMSC adhesion and growth on tissue culture plates at 24 hr of cell co-culture with Mg-xZn-0.5Ca (x = 0.5, 1, 2, 4 wt. %) compared with pure Mg (PMg) control and positive control (cells only group was not incubated with a metallic sample); scale bar = 100 µm for all images. (b) BMSC adhesion density on the surface of each Mg-xZn-0.5Ca alloy, PMg, and on the tissue culture plate surrounding the samples and without sample (cells only positive control) at 24 hr of cell culture (mean ± SEM; n=3; no statistically significant differences were found among the groups at 24 hr of culture). (c) Scanning electron microscopy images (with color indicating elemental map from energy dispersive x-ray spectroscopy) of single BMSC adhesion on the surface of each Mg-xZn-0.5Ca alloy and pure Mg control; scale bar = 5 µm for all images.