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ROLE OF TITANIUM-WOLLASTONITE IN PROMOTING MESENCHYMAL STEM CELLS GROWTH

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SUMMARY

Titanium and wollastonite have been fabricated using powder mixing with 90% of the powder being titanium and 10% wollastonite followed by sintering. Another composite composed of titanium and hydroxyapatite was also produced in this way to determine which one is superior to the other. Three controls materials namely titanium, hydroxyapatite and wollastonite were also developed to document comparisons. Cell viability on the materials were conducted using PretoBlue and results have shown that the composite scaffolds support better cell viability and proliferation than their counterparts. We have concluded that titanium ceramic composites have outperformed the control materials in every way and among each other, titanium wollastonite has proven to be significantly better than titanium hydroxyapatite.

1.0 Introduction

Titanium implants have shaped the implant industry for many years because of its inertness which does not trigger an immune response for implants. Although titanium is regarded for its ability to not produce any negative reactions to the body, it does not promote positive attributes either. Therefore, we propose ceramic to be introduced as ceramic contain bioactive properties; properties that promote cell differentiation and osseointegration between bone and material. Wollastonite which is a ceramic that has been derived from rice husks contains these properties that help

stimulate osteogenesis. Furthermore, wollastonite is potentially a cheaper alternative to hydroxyapatite, a common ceramic used in the clinic as bone fillers or implant coatings. Hence, this study evaluated and compared the biocompatibility of the two titanium-ceramic scaffolds.

2.0 Materials and Method

Titanium powder (Ti6Al4V; Grade 5; TLS Technik GmbH & Co Spezialpulver KG, Germany) and hydroxyapatite (Sigma-Aldrich, USA) were purchased while wollastonite was synthesized using an in-house technique[1]. Titanium alone, ceramic alone and titanium ceramic composite (9:1, w/w) were fabricated in the form of a disc using hot compression molding and vacuum sintering method.

Human mesenchymal stem cells (MSCs) were isolated from bone marrow from patients who were undergoing surgery for the correction of scoliosis. Cells were cultured and expanded in vitro in cell culture medium comprised of α-MEM along with a 10% fetal bovine serum until Passage 3 for the subsequent tests performed in this study.

2.1 Cellular Proliferation Test

Bone marrow derived MSCs were seeded onto the scaffolds and immersed in cell culture medium comprised of α-MEM along with a 10% fetal bovine serum. PrestoBlue™ (Invitrogen, USA), a cellular viability agent was used to determine the viability of cells that were seeded onto the scaffolds at Day 1, Day 5 and Day 7. Absorbance at 570nm was read using a spectrophotometer (Biotek, USA)

2.2 Live-Dead Staining for Cell Viablility

Live-Dead staining kit (BD, USA) was used to identify cellular viability by observing cells stained with a green and red dye by which later it is observed under a fluorescence microscope. Cells stained green observed under the microscope represented live cells and a cells stained red were dead or dying cells. Figure 2 shows the images obtained from staining the cells with the kit. During the observation under live microscopy (Nikon, Japan) over 48 hours, migration of cells on the material was recorded as a video and evaluated using the software (Image Pro, Nikon, Japan).

3.0 Results

Figure 1 shows the fabricated titanium or titanium ceramic scaffolds used in the study. Further evaluation using scanning electron microscope revealed that apatite formation occurred on both titanium-hydroxyapatite and titanium-wollastonite scaffolds but not titanium scaffold when the scaffolds were immersed in the culture medium (images not shown).

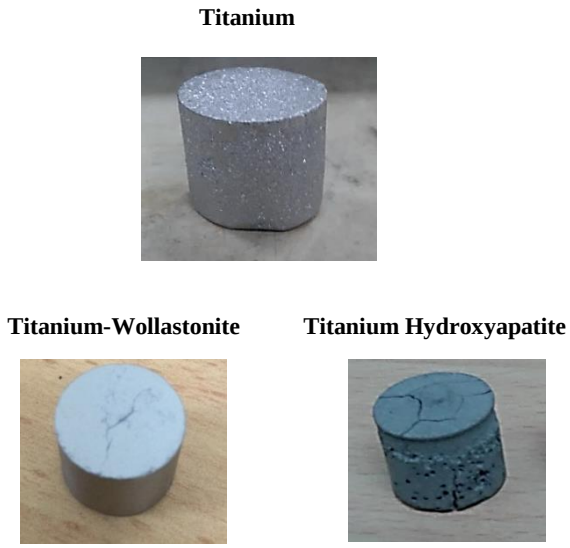

Fig 1: Titanium and titanium-ceramic composites scaffolds

3.1 Cell Proliferation and Viablility

Cell proliferated in titanium only, ceramic only and titanium-ceramic scaffolds. Figure 2 shows the results of PrestoBlue assay obtained on Day 7.

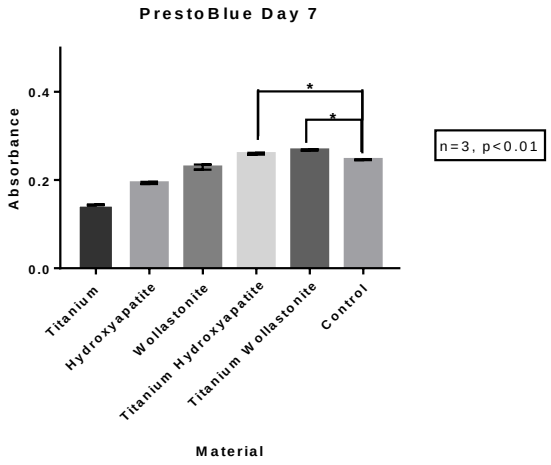

Fig 2: Cell Proliferation on Scaffold Surface after 7 days

3.2 Live-Dead Imaging for Scaffold Samples

Imaging under a confocal scanning microscope (Nikon A1, Japan) confirmed the adherence of cells on the titanium, ceramic and titanium-ceramic scaffolds. Figure 3 shows the

images taken using confocal scanning microscope at Day 7 post-seeding of cells on the scaffolds.

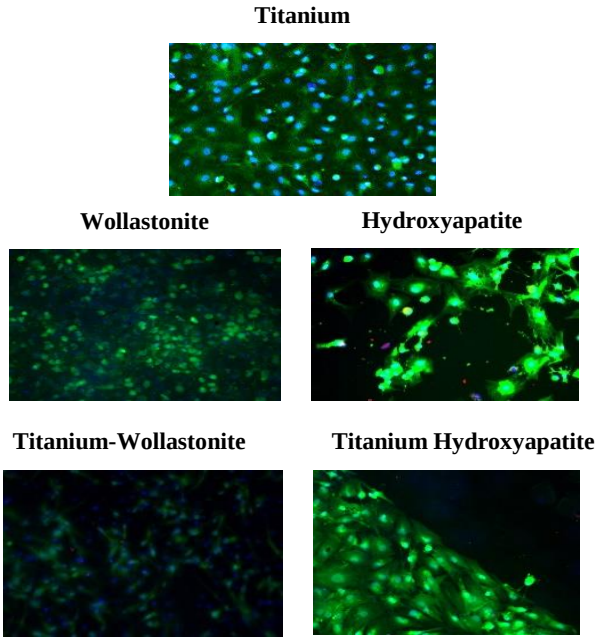

Fig 3. Cell viability at Day 7 post-seeding detected using Live-Dead cell staining kit. Green stained cells are live cells and red stained cells are dead cells

4.0 Discussion & Conclusion

Our results revealed that the fabricated titanium-ceramic scaffolds are not cytotoxic and even better supported cell growth. The proliferation and cell viability on titanium wollastonite and titanium hydroxyapatite were significantly higher than that of the control at Day 7 ($p \leq 0.01$). Cell viability on titanium-wollastonite was slightly higher than that of titanium hydroxyapatite. This could be due to the cells preference to the apatite layer generated by both scaffolds whereby the amount produced by titanium wollastonite is more than that of titanium hydroxyapatite. For the case of the Live-Dead imaging, here we can see that there are viable cells in every scaffold indicating that they are viable to attach for at least 7 days. Cells remained static on titanium alone. Cells migration was also more apparent in titanium-wollastonite (data not shown). This further verified that titanium – wollastonite is potentially more bioactive. In conclusion titanium wollastonite has proved to be fair contender with titanium hydroxyapatite as both scaffolds have been proven to be viable for cell attachment, proliferation and sustained cell viability. The composite is proposed to be used as permanent bone implant due to its higher bioactivity and thus the predicted better osteointegration with host bone. Evaluation of wollastonite-induced osteogenic-related gene expression by MSCs is now underway.

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