Developing a Novel Methodology for Microwave Hydrothermal Nanomodification of Titanium Microcarriers for Bone Regeneration

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Introduction

Dental and orthopedic implants made of titanium and its alloys, despite their decades-long research trajectory and success (1)(2), do not fall within the strict definition of tissue engineering. Still, their study has allowed us to better understand the biological processes that occur at the cell-biomaterial interface and the mechanisms involved in bone repair (1).

The hierarchical combination of micro- and nano-scale roughness can provide the physicochemical and structural properties necessary for stem/progenitor cell osteoblastic differentiation that can lead to bone repair (3). However, bulk implants continue to represent an invasive, “replacement” technology.

We aim to rethink decades of knowledge generated on the modified surfaces of bulk titanium implants and apply it in the development of a new bone regenerative therapy based on nanostructured titanium microcarriers that offers the benefits of an injectable, minimally invasive and localized technology.

The present study establishes a novel methodology for the nanomodification of these titanium microcarriers and their characterization.

Methods

Titanium alloy (Ti6Al4V) microparticles (Ø=150-200 µm) (TI016015/1, Goodfellow Cambridge Ltd., Huntingdon, England) were analyzed as received and after being treated with a microwave hydrothermal (MWHT) nanomodification protocol. The MWHT treatment is performed at low temperatures (100-200°C) in a controlled oxidative environment (i.e., 2.5M H2O2) using a Mars 6 synthesis microwave (CEM Corporation, Matthews, NC, USA) for up to 2 hours. In this study we evaluated the following conditions: 200°C for 2 h, 150°C for 2 and 1.5 h, and 100°C for 1 h. After the treatment, the supernatant is decanted and the processed material is transferred to a microcentrifuge tube, centrifuged for 2 minutes and the pellets are dried in a vacuum desiccator. The surface morphology and chemistry of the nanostructured microcarriers was characterized using a Quattro S scanning electron microscopy (SEM) (Thermo Scientific, Waltham, MA, USA) and image analysis software (ImageJ, Bethesda, USA), as well as energy dispersive X-ray spectroscopy (EDX).

Discussion

Initially, we performed the MWHT nanomodification of the titanium microcarriers based on a protocol already published on solid titanium disks, using 200°C for 2 h. The processed samples were analyzed by SEM and exhibited the presence of well-defined nanostructures on the surface, similar to what was expected from our previous work. However, we found that some particles showed evidence of fragmentation and loss of their spherical architecture, which could generate smaller debris that may be internalized by cells. Thus, we decided to explore other conditions that could minimize this effect, while retaining the surface nanoroughness.

Subsequent tests were focused on reducing temperature and time of modification, as the possible variables that could have an impact on particle fragmentation. Indeed, MWT treatments using 100 ºC had little effect on the presence of nanostructures on the surface, while 150 ºC for 1.5 and 2 h achieved consistent nanomodification with no evident fragmented particles.

Conclusion

- Temperature (T) and exposure time (t) during the MWHT treatment play a critical role in defining the surface nanostructure and integrity of the particles at a constant pressure.
- Nanostructure density and size seemed to be directly correlated with T and t, with the treatment at 200°C for 2 h generating the most noticeable nanostructured surface.
- However, we found a sweet-spot for the generation of nanomodifications with the lowest impact on particle fragmentation using parameters of 150°C and 1.5 h.
- The MWHT nanomodification treatment works through a controlled-oxidation process, and chemical analysis using EDX seems to confirm this by showing a considerable increase in the content of elemental oxygen on the treated surfaces.

Results

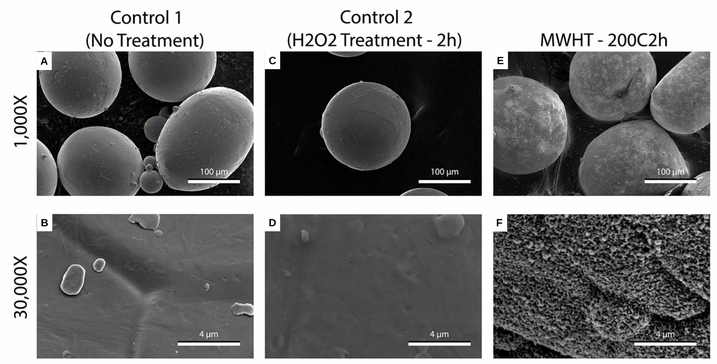


Figure 1. Electron micrographs of Ti6Al4V microcarriers at low and high magnification. SEM images were collected from samples: (A, B) as received (Control 1), treated only with H2O2 but without microwave (Control 2) or MWHT-treated at 200°C for 2 h. The presence of a dense layer of nanostructures on the surface of the MWHT-treated samples was evident.

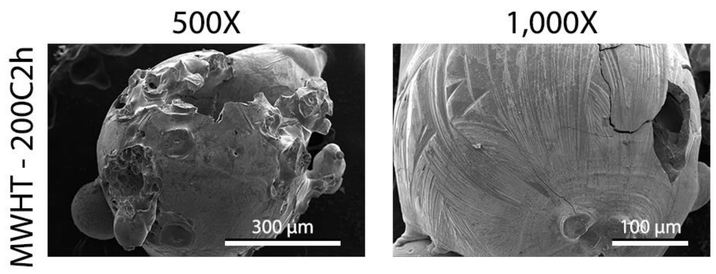


Figure 2. Evidence of microcarrier fragmentation. Electron micrographs of MWHT-treated samples at 200°C for 2 h showed evidence of particle fragmentation.

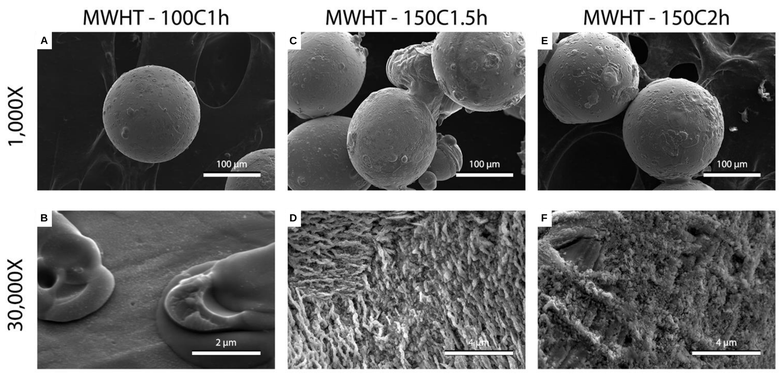
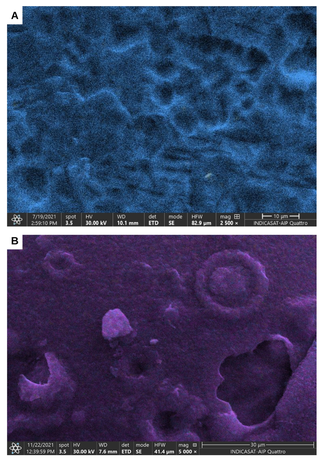


Figure 3. Electron micrographs of Ti6Al4V microcarriers MWHT-treated with different parameters. Ti6Al4V microcarriers were MWHT-treated with the following parameters: (A, B) 100°C for 1 h, (C, D) 150°C for 1.5 h and (E, F) 150°C for 2 h. The presence of fragmented particles was not evident when the temperature was at or below 150°C for 1.5 h or less. However, the samples treated with 100°C for 1 h did not show any visible changes.



C

	Element [wt %]				
	C	O	Al	Ti	V
Control	0.3	0	0.2	99.5	-
MWHT	1.3	19.5	5.2	70.9	3.1

Figure 4. ColorSEM images and EDX analysis of Ti6Al4V microcarriers. (A) As received and (B) MWHT-treated Ti6Al4V microcarriers were analyzed with an EDX detector. The ColorSEM images (A, B), show that the distribution of the elements detected was homogeneous throughout the surface. (C) The EDX results shown on the table suggest that the MWHT treatment occurs through an oxidation process at the surface.

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