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Original Article

Biomedical Ti–Cu–Mn alloys with antibacterial capability

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ABSTRACT

This study aims to develop Ti–Cu–Mn alloys with antibacterial capability for reducing the possibility of bacterial infection during biomedical implant surgeries. Ternary alloys were designed to be manufactured via powder metallurgy, which has intrinsic manufacturing, economic, and environmental benefits. The amount of each of the alloying elements was limited to prevent the formation of brittle phases. It is found that the selected compositions of Cu (0.5-5 wt.%) plus Mn (0.25-2.5 Mn wt.%) can successfully be manufactured achieving microstructures, tensile properties and antibacterial capability performance comparable to those of other biomedical Ti alloys. Specifically, homogeneous compositions are achieved with the selected sintering parameters (1300 °C/120 min). The strength and hardness of the material increase proportionally with the addition rate of Cu and Mn, and antibacterial capability values were found to be above the 90% threshold required for antibacterial certification.

1. Introduction

Ti alloys, as well as other metallic materials like stainless steel and CoCr alloys [1,2], are commonly used in the field of biomedicine as prosthetic materials for the replacement of failed joints such as hip and knee. This is because metals can provide the much needed mechanical performance and endurance for long term implants. As biomedical materials, Ti alloys are generally favoured due to their combination of properties including biocompatibility, and relatively low modulus of elasticity [3,4]. Most metallic biomaterials are, at best, inert with human body fluids. Surface modification to reduce the rejection of the prosthesis by the human body is a common practise [5]. The two most widely adopted Ti alloys for biomedical applications are pure Ti and the Ti-6Al-4V alloys where the latter is preferred for structural prostheses due to its higher mechanical performance. However, reports about the cytotoxicity as well as other harmful effects such as neurotic effect [6] of V [7] and Al [6,8,9] are available in literature.

Formulation of new Ti-based biomedical alloys offers the possibility to design compositions entailing biocompatible elements with limited cytotoxicity. Moreover, smart design of novel alloys could also consider other aspects, namely the high cost of Ti alloys and the functionalisation of the material with properties that would not otherwise be achieved if not by means of further processing. Referring to the latter, pathogenic infection of biometallic prosthesis is a rising issue [10],

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Table 1 – Features of the Ti–Cu–Mn alloys studied.

Material	Cu [wt.%]	Mn [wt.%]	Ti [wt.%]	Theoretical density [g/cm^3]
Ti-0.5Cu-0.25Mn	0.5	0.25	99.25	4.54
Ti–1Cu-0.5Mn	1	0.5	98.5	4.57
Ti–2Cu–1Mn	2	1	97	4.63
Ti-3.5Cu-1.75Mn	3.5	1.75	94.75	4.72
Ti–5Cu-2.5Mn	5	2.5	92.5	4.80

which leads to the failure of the permanent implants [11]. Therefore, newly designed compositions could consider the addition of antibacterial elements to prevent pathogenic bacterial infection. From literature, Sn [12], Ag [13] and Cu [14] are elements with antibacterial capability. Sn has very low melting point and this causes problems during manufacturing, whilst Ag is an expensive element, which will further increase the cost of Ti-based biomaterials. The use of Cu as alloying element in Ti alloys to provide antibacterial capability has recently been investigated. The majority of the work performed considered binary Ti–Cu alloys [15] or the modification of already existing Ti alloys such as Ti–6Al–4V [16]. Manufacturing binary Ti–Cu alloys with a Cu content of up to 25 wt.% via hot pressure sintering, Liu et al. [15] demonstrated that the addition of Cu leads to the formation of Ti_2Cu particles and the release of Cu^{2+} ions able to kill bacteria. Regarding the modification of existing Ti alloys, both Aoki et al. [17] and Ren et al. [18] reported the modification of the Ti–6Al–4V with the addition of Cu, up to 10 wt.%. Both studies showed that cast Ti–6Al–4V + Cu alloys have antibacterial capability. Although not used for antibacterial response, the use of Mn as alloying element as also been investigated. Santos et al. [19] developed binary Ti–Mn alloys as Mn plays a key role in osteogenesis and bone resorption mechanisms [20], as well as other essential mechanisms for regular body functions [21].

Both Cu and Mn are cheap and commercially available chemical elements able to stabilise the BCC β Ti phase, which could be used to enhance the bio-related properties of Ti alloys. Moreover, they improve the mechanical properties of Ti via different strengthening mechanisms. Lowering the specific cost of the material can be achieved especially if the manufacturing of such Cu and Mn bearing alloys is performed using powder metallurgy (PM). This is because PM allows to obtain near net shape products with a reduced amount of processing steps, limited machining operations, and lower power consumption. Furthermore, PM consents to easily change the chemical composition through the addition of elemental powders via the so called blended elemental approach [22].

The aim of the current study is thus to investigate the development of Cu and Mn bearing PM Ti alloys. Specifically, powder mixtures of Ti alloys with different Cu + Mn addition rates were designed to be produced via blended elemental powders and converted into solid materials via cold uniaxial pressing plus vacuum sintering. The alloys developed were tested to quantify their microstructure, porosity level, mechanical performance and *in vitro* antibacterial activity against *Escherichia coli* (*E. coli*). The purpose was to prove that they can be successfully implemented as structural biometallic prostheses with the added functionality of antibacterial capability.

2. Experimental procedure

Ti–Cu–Mn alloys were obtained by blending elemental Ti (D_{90} < 75 μm, 99.4% purity, irregular morphology, Goodfellow Ltd.), Cu (D_{90} < 63 μm, 99.7% purity, dendritic morphology, Merck KGaA) and Mn (D_{90} < 45 μm, 99.0% purity, angular morphology, Sigma–Aldrich Ltd.) powders. The total amount of alloying elements was chosen on the basis of their β stabilising effect and the Ti–Cu and Ti–Mn binary phase diagrams [23] to limit the formation of unwanted phases such as the brittle ω Ti phase. The details of the compositions studied and some of their features are shown in Table 1. The theoretical density of the alloys was calculated through the rule of mixture using the density of the pure elements. The powder mixtures were mechanically blended for half an hour for their cold compaction at 600 MPa and subsequent sintering at

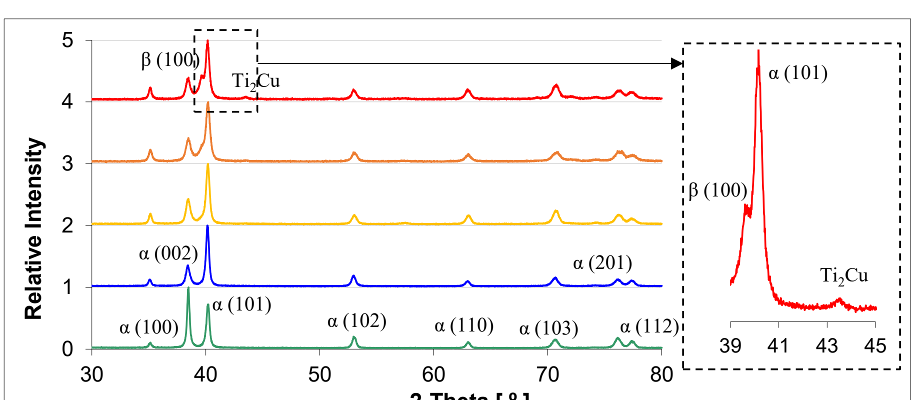

Fig. 1 – Results of the XRD analysis performed on the PM Ti–Cu–Mn alloys. Note: 0) Ti-0.5Cu-0.25Mn, 1) Ti–1Cu-0.5Mn, 2) Ti–2Cu–1Mn, 3) Ti-3.5Cu-1.75Mn, and 4) Ti–5Cu-2.5Mn alloys.

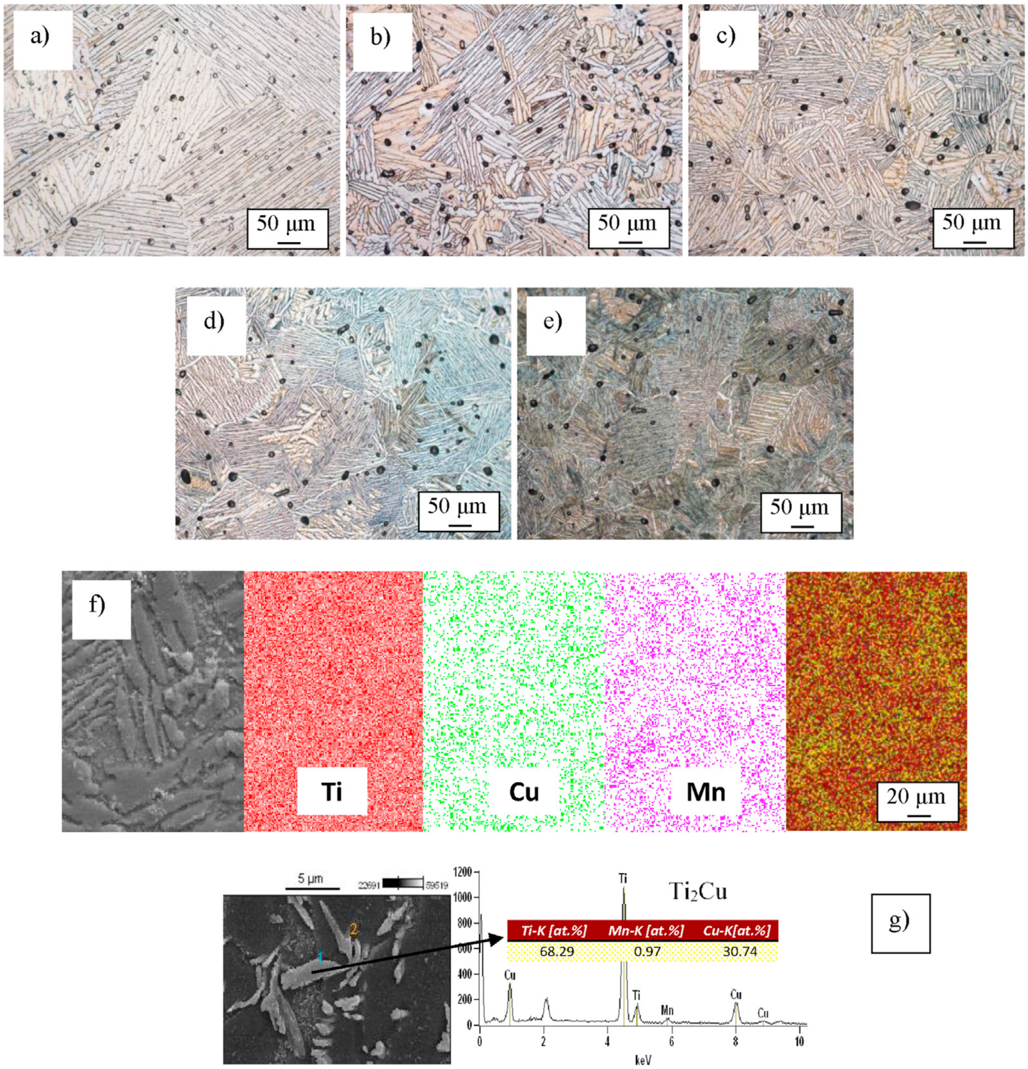

Fig. 2 – Representative results of the microstructural analysis performed on the PM Ti–Cu–Mn alloys including optical micrographs of the: a) Ti-0.5Cu-0.25Mn, b) Ti–1Cu-0.5Mn, c) Ti–2Cu–1Mn, d) Ti-3.5Cu-1.75Mn, and e) Ti–5Cu-2.5Mn alloys; f) elemental mapping analysis (Ti–5Cu-2.5Mn alloy), and g) detail of the hypoeutectoid structure of the Ti–5Cu-2.5Mn alloy with EDS analysis of Ti_2Cu particles.

1300 °C for 120 min under a vacuum level of 10^{-3} Pa. The heating and cooling rates were set at 10 °C/min.

X-ray diffraction (XRD, X'pert Malvern Panalytical) analysis was done with scanning step 0.013° between 30 and 80°. Samples for microstructural characterisation were cut, ground (SiC), polished (colloidal silica), and chemically etched using a standard Kroll reagent. The microstructure was analysed by optical (Olympus BX 60) and scanning electron (Hitachi S 4700) microscopy. The density of the samples before (viz. green) and after sintering was obtained via calculation (mass/volume ratio) and by means of water displacement measurements, respectively. The porosity level is the difference between the density of the full dense alloy (i.e. theoretical density) and the sintered material. A minimum of five Rockwell hardness (HRA) measurements were performed to quantify the hardness of the materials. At least three dog-bone tensile samples (2 × 2 mm^2 cross section and 20 mm gauge length) were tested using a cross-head test speed of 0.1 mm/min to obtain the tensile behaviour of the alloys by means of an Instron 33 R 4204 equipment.

To quantify the antibacterial capability, the Ti–Cu–Mn alloys were *in vitro* tested against *E. coli* (*E. coli*, Life Technologies Ltd.). The JIS2801:2010 standard [24] with minor modifications was followed to perform the plate count method. Triplicate samples with 30 μL of inoculum were incubated at 37 °C for 24 h and afterwards the extracted bacteria were serially diluted up to 10^{-6} through the addition of 100 μL of PBS (Phosphate-buffered saline) solution. The antibacterial capability was obtained by means of the commonly used colony forming units (CFU) counting.

3. Results and discussion

From the XRD spectra of the Ti–Cu–Mn alloys shown in Fig. 1, it can be seen that different phases are found as a function of

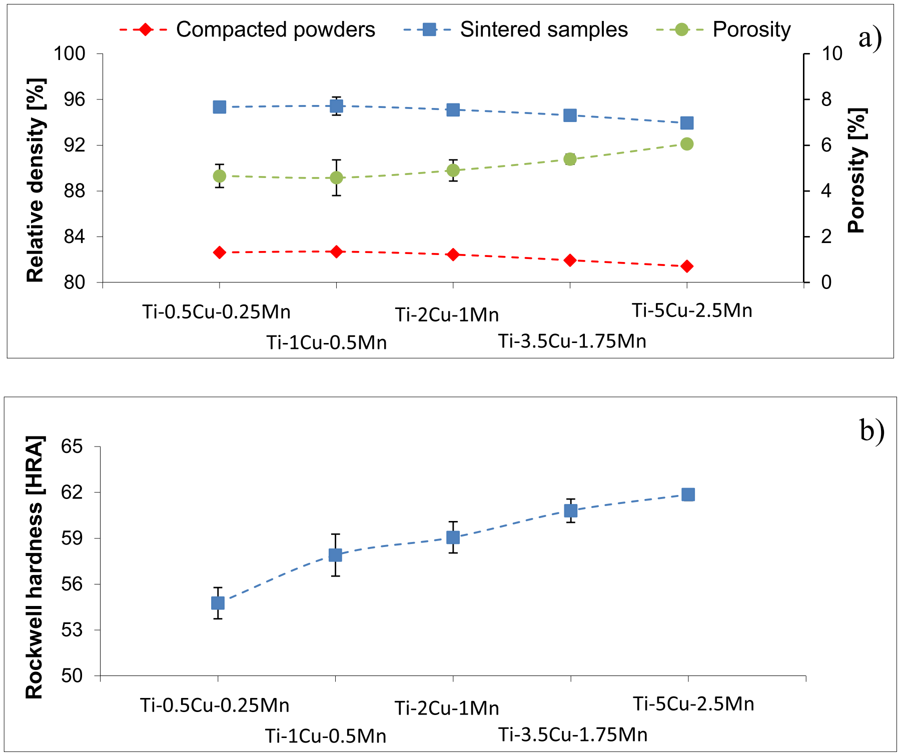

Fig. 3 – Results of the density (a) and hardness (b) measurements performed on the PM Ti–Cu–Mn alloys.

the total amount of alloying elements. The major phase of the Ti–Cu–Mn alloys is always the α Ti phase regardless of the alloy composition considered. Although both Cu and Mn are stabilisers of the β Ti phase, the primary peak of the β Ti phase is not detected when its amount is small, which is typical for the so called near α Ti alloys [25]. As the amount of stabilised β Ti phase increases, due to the presence of a higher content of alloying elements, the primary peak of the β Ti phase becomes more pronounced. Once the content of Cu added reaches 2 wt.%, precipitation of the tetragonal Ti_2Cu intermetallic phase in the microstructure occurs [23] and thus the corresponding primary peak is detected in the XRD spectra of Fig. 1. XRD analysis confirms that the formation of Mn-based intermetallic particles as well as that of the brittle ω Ti phase, which can potentially be found in Ti–Mn alloys [19], is effectively prevented by limiting the amount of Mn in the PM Ti–Cu–Mn alloys.

Fig. 2 shows representative results of the microstructural characterisation performed on the PM Ti–Cu–Mn alloys. The materials have the typical structure of slow cooled α+β Ti alloys composed of prior β Ti grains and α+β lamellae. Officially, the alloys with a total amount of β stabilisers alloying elements lower than 2–3 wt.% (i.e. Ti-0.5Cu-0.25Mn and Ti–1Cu-0.5Mn) are classified as near α Ti alloys. They are characterised by much coarser microfeatures and a significantly smaller amount of retained β Ti phase. The size of the prior β Ti grains is slightly refined but not greatly affected by the composition as it is more dependent on the processing route and all the PM Ti–Cu–Mn alloys were sintered under the same conditions. Nevertheless, the amount of Cu and Mn critically changes the amount of retained β Ti phase in the microstructure leading to the formation of finer microstructure as the amount of alloying elements increases.

As it could be expected from literature about sintering of blended elemental titanium alloys processed above the β transus of the material [26,27], the PM Ti–Cu–Mn alloys are characterised by the presence of pores that could not be eliminated during the sintering process. These micrometric-size pores are regularly dispersed in the microstructure. They are found both within and at the grain boundaries of the prior β Ti grains, and have a round shape. These features indicate that the third stage of sintering was reached with the processing conditions employed (i.e. 1300 °C/120 min). As confirmed via EDS elemental chemical analysis, the PM Ti–Cu–Mn alloys have a homogeneous distribution of alloying elements. For instance, the average EDS chemical composition of the most heavily alloyed Ti–5Cu-2.5Mn composition is: Cu = 5.93 ± 0.88, Mn = 2.51 ± 0.26, and Ti=Balance. A fully homogeneous composition was detected in the most heavily alloyed material (Fig. 2f), as so was for the other PM Ti–Cu–Mn alloys. As β Ti stabilisers elements with reduced solubility in the α Ti phase, Cu and Mn are more heavily encountered in the β Ti phase and therefore within the β Ti lamellae. SEM analysis also showed that, for alloys where the added Cu reaches 2 wt.%, the Ti_2Cu intermetallic phase precipitates at the α+β lamella boundaries. The typical hypoeutectoid structure is found at the grain boundaries of the Ti–5Cu-2.5Mn alloy (Fig. 2g).

Fig. 3 shows the results of the measurements of the density and hardness of the PM Ti–Cu–Mn alloys. The density of the compacted powders decreases somewhat as the content of the alloying elements increases. Due to their morphology, the

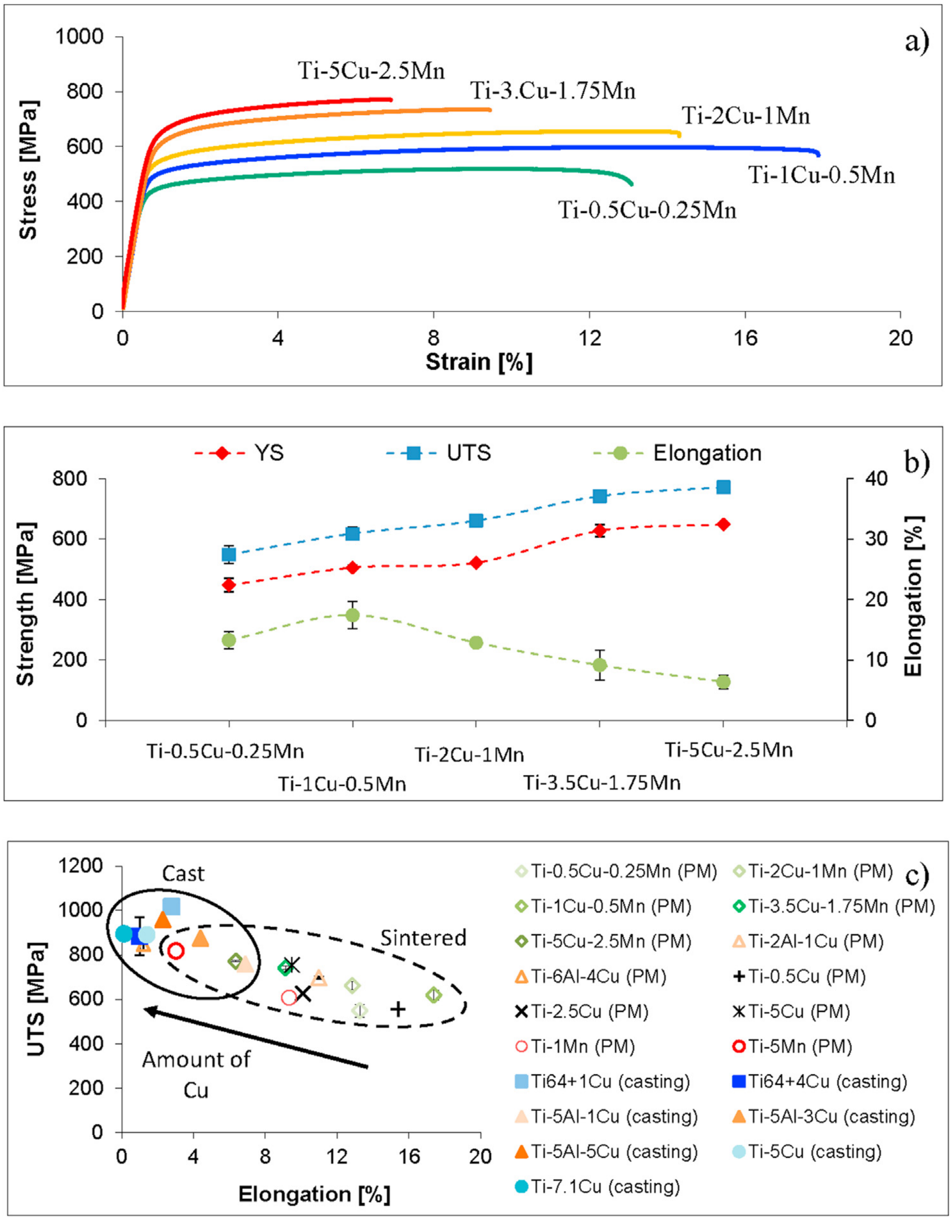

Fig. 4 – Results of the characterisation of the tensile properties of the PM Ti–Cu–Mn alloys: a) representative tensile curves, b) average yield strength, ultimate tensile strength and elongation, and c) comparison with literature [17,29–33].

three elemental powders used as starting materials are well-suited to be processed by means of PM. However, their combination and the difference in hardness leads to the density of the pressed powder to decrease from 82.6% to approximately 81.4%. It can be noticed that this affects the density of the sintering alloys, which slightly decreases with the addition of the alloying elements. Nevertheless, the variability within the range of the values obtained (94–95%) is small. The associated amount of residual porosity has the opposite trend of the density of the sintered alloys. It slightly increases within the 4–5% range.

The value of relative density achieved in each alloy (Fig. 3) is the result of the balance between the green density and the diffusion of the alloying elements within the titanium base. In particular, both Cu and Mn have higher diffusivity rates than that of Ti self-diffusion [28]. Thus, they improve the sold state sintering response of Ti. It is found that the difference between the green density and the sintered density for each alloy is similar (viz. 12.7 ± 0.1%) regardless of the total amount of alloying elements added. The relative density values achieved are therefore a direct consequence of the starting green density value, which slightly decreases as more alloying elements are present in the powder blend. Whereas the densification associated with the diffusion of the alloying elements is constant.

From Fig. 3b, the hardness of the PM Ti–Cu–Mn alloys increases continuously with the progressive addition of Cu and Mn as the lamellar structure is refined. Moreover, a higher

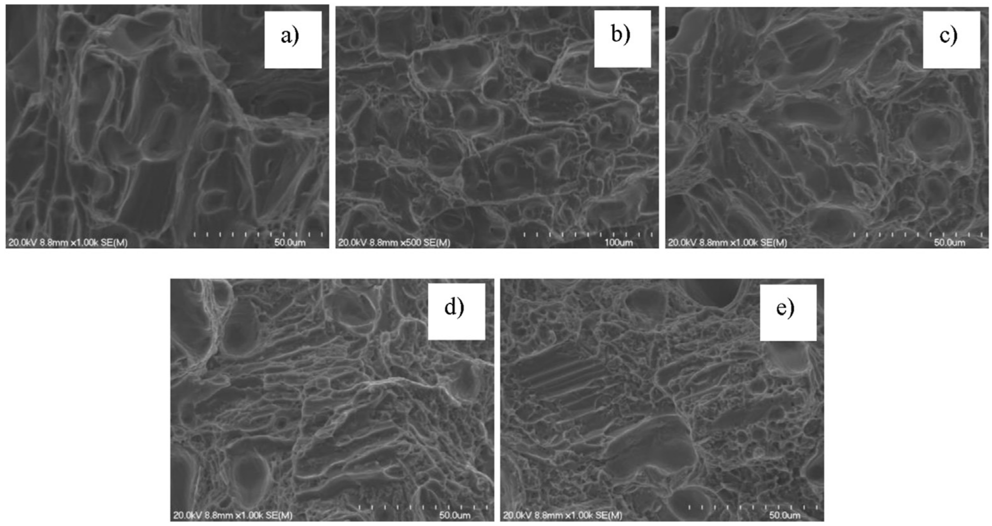

Fig. 5 – Results of the fractographic analysis performed on the PM Ti–Cu–Mn alloys: a) Ti-0.5Cu-0.25Mn, b) Ti–1Cu-0.5Mn, c) Ti–2Cu–1Mn, d) Ti-3.5Cu-1.75Mn, and e) Ti–5Cu-2.5Mn.

amount of β Ti phase is retained upon cooling from the sintering temperature, and the alloying elements strengthen the Ti matrix. The hardness increases linearly from 55 HRA to 62 HRA without a greater improvement when the Cu content is ≥ 2 wt.% meaning that the number of precipitated Ti_2Cu intermetallic particles is fairly low and their size relatively small.

The representative tensile curves of Fig. 4a show that the strength of the PM Ti–Cu–Mn alloys linearly increases and, with the exception of the Ti-0.5Cu-0.25Mn alloys, the ductility linearly decreases as more Cu and Mn are added to Ti. Moreover, the yield point is shifted to higher values as the total amount of alloying elements increases but the modulus of elasticity is very similar among the different PM Ti–Cu–Mn alloys. More in detail, the yield stress ranges from 450 MPa to 650 MPa, progressively increasing with the amount of alloying elements. The ultimate tensile strength ranges from 550 MPa to 770 MPa, progressively increasing with the amount of alloying elements. The elongation at fracture ranges between 6.4% and 17.4%. For the latter, the lowest value is obtained for the Ti–5Cu-2.5Mn alloy and the highest for the Ti–1Cu-0.5Mn alloy with the other alloys having intermediate values. The progressive strengthening and embrittlement of the PM Ti–Cu–Mn alloys is due to the balance between the level of residual porosity (Fig. 3), the size and characteristics of the phases present in the microstructure (Fig. 2), which become finer for higher amount of alloying elements, and the contribution of the substitutional solid solution strengthening of the Cu and Mn atoms. The majority of these mechanisms hinder the movement of dislocations justifying the increase in strength and decrease in ductility. Furthermore, the precipitation of the brittle Ti_2Cu intermetallic phase, which becomes more relevant as a greater amount of alloying elements are added, contributes to the reduction of the ductility (Fig. 4b).

The average tensile properties of the PM Ti–Cu–Mn alloys are comparable to those of the sintered binary Ti–5Cu alloy (yield strength of 627 MPa, ultimate tensile strength of 754, and elongation at fracture of 9.5% [30]). They also have lower strength but higher ductility than the sintered binary Ti–5Mn alloy (yield strength of 716 MPa, ultimate tensile strength of 817, and elongation at fracture of 3.0% [31]). The properties reported in Fig. 4b are higher with respect to those of the binary Ti–5Cu alloys obtained through casting (yield strength of 570 MPa, ultimate tensile strength of 630 MPa, and elongation at fracture of 3.2% [34]). From Fig. 4c, for low alloying elements addition rates and independently of the processing route, the sintered Ti–Cu-Mu alloys generally show lower strength but higher ductility with respect to other antibacterial Ti-based alloys. The Ti–5Cu-2.5Mn alloy has comparable properties to those of the cast Ti–5Al–1Cu alloy [33]. Moreover, in general, the alloys obtained via PM have slightly lower strength but much better ductility than cast binary Ti–Cu alloys [17,32].

Fig. 5 shows the results of the fractographic analysis performed on the fracture surface of the tensile test pieces made out of the different PM Ti–Cu–Mn alloys. It is found that the fracture surface is primarily composed of dimples, typical of the behaviour of ductile metals. However, the relative amount of intergranular fracture features increases for higher alloying elements addition as a consequence of the embrittlement of the alloy. Specifically, intergranular fracture is found at the boundaries between the α Ti and β Ti lamellae as clearly visible in Fig. 5e.

Fig. 6 presents the results of the *in vitro* antibacterial capability assessment where it is shown that the number of *E. coli* colonies that survived after 24 h of inoculation on the surface of the PM Ti–Cu–Mn alloys decreases with the amount of alloying elements. More in detail, the materials with an alloying content ≤3 wt.% have an antibacterial capability of 89%, which is slightly lower than 90%. According to the GB/T 4789.2-2010 standard [35], 90% is considered to be the minimum value for materials with antibacterial capability. From Fig. 6e, the PM Ti–Cu–Mn alloys have however better

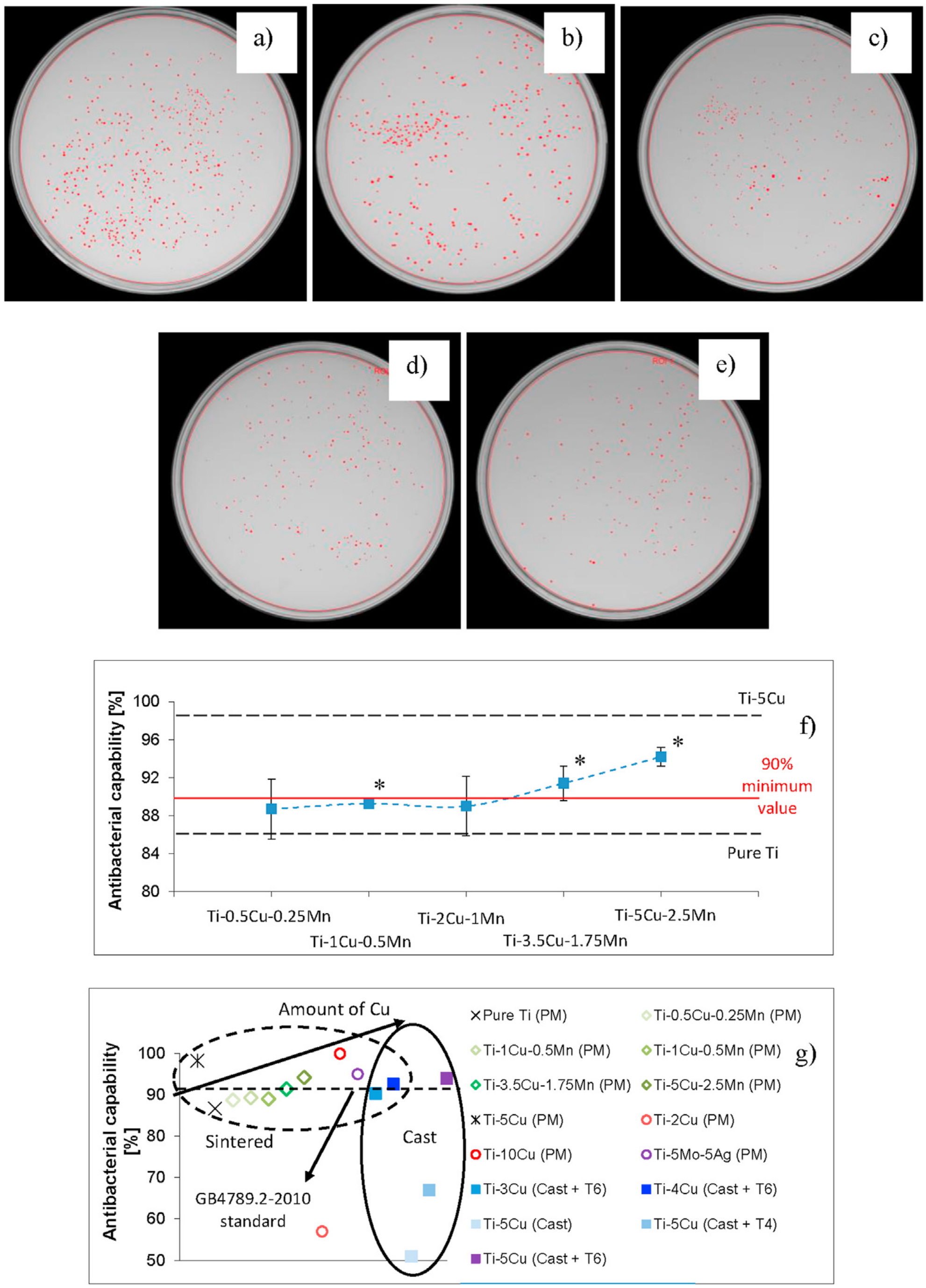

Fig. 6 – Results of the *in vitro* antibacterial capability assessment including representative photos of distribution of the *E. coli* colonies for: a) Ti-0.5Cu-0.25Mn, b) Ti–1Cu-0.5Mn, c) Ti–2Cu–1Mn, d) Ti-3.5Cu-1.75Mn, and e) Ti–5Cu-2.5Mn, f) average antibacterial capability, and g) comparison with literature [15,36–38]. *$p < 0.05$.

antibacterial response with respect to sintered Ti (86.6%), which was used as negative control, and lower response compared sintered Ti–5Cu (98.2%), which was used as positive control.

The performance of the Ti-3.5Cu-1.75Mn and Ti–5Cu-2.5Mn alloys in terms of antibacterial capability is comparable to that of other Cu-bearing titanium alloys available in literature. Specifically, antibacterial rates of 90.3% and 92.6% for, respectively, the cast and T6 heat treated Ti–3Cu and Ti–4Cu alloys were reported by Zhang et al. [39]. Antibacterial rates of 51%, 67% and 94% were found for the as-cast Ti–5Cu, T4 treated Ti–5Cu, and T6 treated, respectively, in the study of Zhang et al. [38]. From Fig. 6g, it can be seen that the antibacterial capability is significantly affected by the manufacturing route and a subsequent heat treatment. Generally T6 (i.e. solution plus ageing heat treatment) is needed to achieve antibacterial capability in cast Cu-bearing titanium alloys. This is obviously not the case for the PM Ti–Cu–Mn alloys. The antibacterial capability of the PM Ti–Cu–Mn alloys is also comparable to that of the more costly Ti–5Mo–5Ag alloy [36].

4. Conclusions

From this study about the development of biomedical Ti–Cu–Mn alloys with antibacterial capability via the PM blended elemental approach combined with cold pressing plus vacuum sintering it can be concluded that Ti–Cu–Mn alloys with Cu-to-Mn addition rates of 2-to-1 can successfully be manufactured via PM achieving high levels of relative density, densification as well as homogeneity of the chemical composition. Although there are minor effects from the morphology and nature of the starting powder used, the progressive addition of a greater amount of Cu and Mn to Ti leads to significant changes at microstructural level. This consequently determines the performance of the alloys. Specifically, the higher the amount of Cu and Mn, the higher the amount of retained β Ti phase, the finer the microstructural features of the lamellar structure formed, and the higher the potential to form intermetallic phases such as Ti_2Cu. However, the amount of alloying elements does not significantly affects the porosity. The microstructural changes summarised, combined with the solid solution strengthening effect of the alloying elements, lead to a linear increase of the resistance of the materials (yield strength, ultimate tensile strength and hardness), an inversed U-shaped trend for the ductility of the materials where the highest strain value is obtained in the Ti–1Cu-0.5Mn alloy, and alloys with antibacterial capability.

Data availability

All metadata pertaining to this work will be made available on request.

Declaration of Competing Interest

The authors declare no competing financial interest.

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