In Vitro Biocompatibility Study on Implantable Crystalline Silica-Aluminium Metal-Based Hybrid Composites

Sourav Debnath and Akshay Kumar Pramanick

Abstract Attempt has been taken towards carrying out detailed investigation about the relevance of using fabricated crystalline silica-aluminium metal-based hybrid composite as biomedical implants, in vitro. In vitro corrosion study has carried out into simulated body fluid (SBF), and corresponding changes of surface morphology have found out and reported. In addition, bactericidal analysis and cytotoxicity study have carried out and reported for the same fabricated metal matrix composites as per available standards.

Keywords Crystalline silica-aluminium metal matrix hybrid composite · Surface roughness · In vitro corrosion · Optical microscopy · Bactericidal · Cytotoxicity

1 Introduction

Replacement of natural bone with implants is sometimes essential after severe accident, bone-related diseases, or major tumour removal. Moreover, after a certain age, human are suffering from various joint-related pain and nowadays joint replacement is most common thing. Replacement with artificial implants can relieve the affected patients and improves the quality of living (Learmonth et al. 2007). The used biomedical implants should be biocompatible in nature and will not create any adverse reactions with host tissues. Successful surgery not only depends upon the knowledge of surgeon but also the materials used as implants (Learmonth et al. 2007; Mizuno 2014).

After implantation, implants should direct contact with body fluids and blood tissues. Hence, it is a useful practice to study the behaviour of implantable biomaterials into simulated body fluid, in vitro. In this regard, corrosion test into body fluids (Yang et al. 2006; Abidin et al. 2010; Bidhendi and Pouranvari 2011) is very feasible because corrosion not only the cause of mechanical failure of implantable biomaterials but also may cause tissue inflammations. Hence, corrosion directly hampers the biocompatibility and materials properties.

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Implants should proceed under cytotoxicity and bactericidal analysis for safety assessments (Mizuno 2014; Radu et al. 2008). During last few years, considerable focus has been concentrated on fabricating "tissue trilored" biocompatible implants (Wang et al. 2010; Yamamoto et al. 2007; Tsuchiya et al. 1993; Liu et al. 2015; Vergaro et al. 2010; Nie et al. 2010). This research takes care of this by carrying out cytotoxicity and bactericidal analysis separately.

Present study focused towards fabricating in vitro biocompatible crystalline silica-aluminium metal-based hybrid composites. Several research have carried out on various grads of Mg/Mg-based alloys, Co–Cr alloys, zirconia, titanium, stainless steel or polymer-based implants (Learmonth et al. 2007; Mizuno 2014; Yang et al. 2006; Abidin et al. 2010; Bidhendi and Pouranvari 2011; Radu et al. 2008; Wang et al. 2010; Yamamoto et al. 2007; Tsuchiya et al. 1993; Liu et al. 2015; Vergaro et al. 2010; Nie et al. 2010; Takamori et al. 2008) but little efforts have seen for composites, especially aluminium metal matrix composites with such compositions.

2 Materials and Methods

2.1 Fabrication of Crystalline Silica-Aluminium Metal-Based Hybrid Composite

Crystalline silica-aluminium metal-based hybrid composites have fabricated through "Powder Metallurgy" technique maintaining the steps of milling and mixing, pressing and finally sintering conducted at 600 °C temperature for an hour. In fabricated composites, aluminium matrix is reinforced with crystalline silica by 20 wt% and beta-tricalcium phosphate by 10 wt%. Mentioned that sintering was carried out separately into inert muffle furnace and hot press as described elsewhere (Debnath and Pramanick 2019). Any reaction has not addressed between matrix and hybrid reinforcements for the composite fabricated into hot press (Debnath and Pramanick 2019,2016; Debnath et al. 2016). The prepared metal matrix composite fabricated through hot pressing has preferable physical, mechanical as well as some evaluated in vitro biological properties like bioactivity, haemocompatibility along with satisfactory clot test which is already been demonstrated in our previous study (Debnath and Pramanick 2019).

2.2 Characterization and Biological Evaluations

Characterization and biological evaluations have discussed below.

2.2.1 Microstructure

Crystalline silica-aluminium metal-based hybrid composite was considered for surface morphology analysis before and after in vitro corrosion study, using "LEICA Optical Microscopy, model DM-2700M Image Analyzer."

2.2.2 Surface Roughness

Fabricated crystalline silica-aluminium metal-based hybrid composite was considered for surface roughness measurement as surface roughness has great influence on biocompatibility of implants. Roughness value has measured as per ISO 5436-2 standards employing surface roughness tester, model "Taylor Hobson instrument/Sutronic 3+."

2.2.3 In Vitro Corrosion Test

SBF was prepared followed by "Kokubo's method" (Mizuno 2014; Kokubo et al. 1990). In vitro corrosion study has performed into this SBF medium at identical physiological temperature (36–37 °C), employing "POTENTIOSTAT GALVANOSTAT Super-PG 1000."

2.2.4 Bactericidal Study

Distilled water treated "Nutrient agar media" was autoclaved at temperature 121 °C for the period of 20 min for making it sterile. Mixing with "*Staphylococcus aureus*" has carried out and distributed in the petri dish. Time has provided for solidifying the medium. Fabricated composite under study has considered and poured into the porcelain bit. Due to capillary action, sample has come out into the bit. Used two bits have different concentrations, 2 mg/10 mL and mg/10 mL, which were kept into the same petri dish. At last, the petri dish, carrying the sample under study, has incubated for 24 h, maintaining temperature 30–35 °C.

2.2.5 Cytotoxicity Study

Crystalline silica-aluminium metal-based hybrid composites fabricated into inert muffle furnace and hot press were considered separately for cytotoxicity study. "Prepared peripheral blood mononuclear cells" (PBMC) have incubated with the samples for 24 h. MTT has added and again incubated for four hours. Addition of MTT kills the cells and consequently formation of crystals was found out. "Dimethyl sulfoxide" (DMSO) was used after withdrawn the media. Solution has become purple colour depending upon the amount of formed crystal. Optical density was noted against

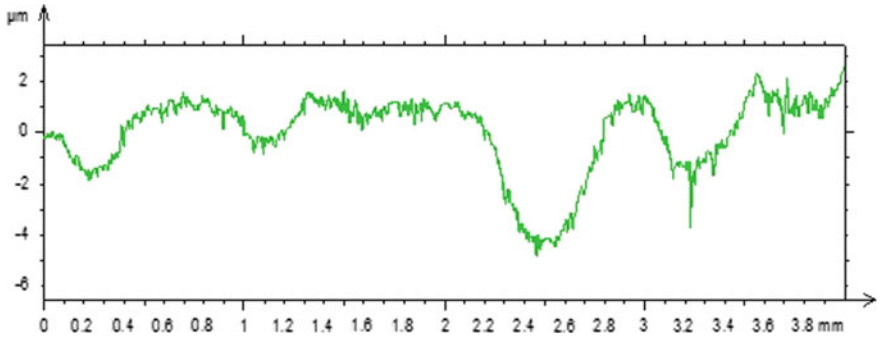

Fig. 1 Surface roughness of crystalline silica-aluminium metal-based hybrid composite

blank when cells are died. Experiment was conducted number of times for each sample under study to get more accuracy.

3 Results and Discussion

Acquired results and consequent discussion are elaborated below.

3.1 Surface Roughness

Figure 1 shows the roughness of fabricated crystalline silica-aluminium metal-based hybrid composite in which arithmetic mean roughness (Ra) was found out 0.405 µm and rms value of surface roughness (Rq) was recorded 0.472 µm. This surface has classified as moderately smooth and useful to proceed biocompatibility evaluations as per ISO/FDIS 23317, Implants for surgery (Mizuno 2014).

3.2 Analysis on In Vitro Corrosion

In vitro corrosion test has performed on fabricated metal matrix composite as shown in Fig. 2 and corresponding I_{corr} and E_{corr} values are recorded in Table 1. It has found out that the value of corrosion is typically low under such test environments.

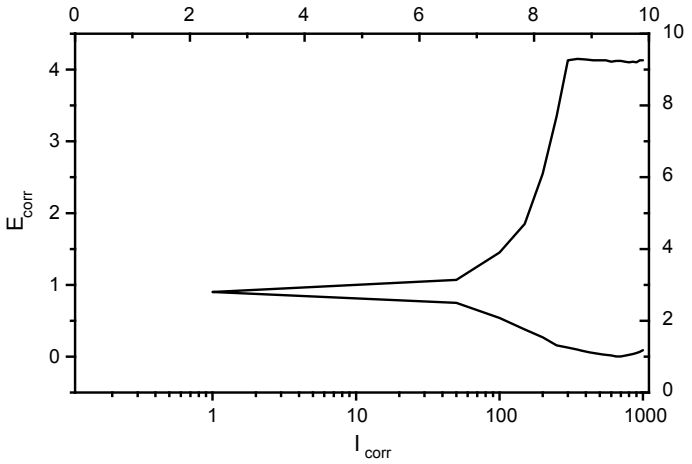

Fig. 2 In vitro corrosion for fabricated crystalline silica-aluminium metal-based hybrid composite into SBF solution

Table 1 In vitro corrosion

Composition	Corrosion current density (I_{corr})	Corrosion voltage (E_{corr})
Crystalline silica-aluminium metal-based hybrid composite	36.808 mA/cm^2	0.885 mV/cm^2

3.2.1 Corroded Surface Evaluation

Figure 3 shows the surface morphology of crystalline silica-aluminium metal-based hybrid composite at different magnifications. In this surface structure, it has observed that reinforcing phases are distributed systematically into aluminium metal matrix.

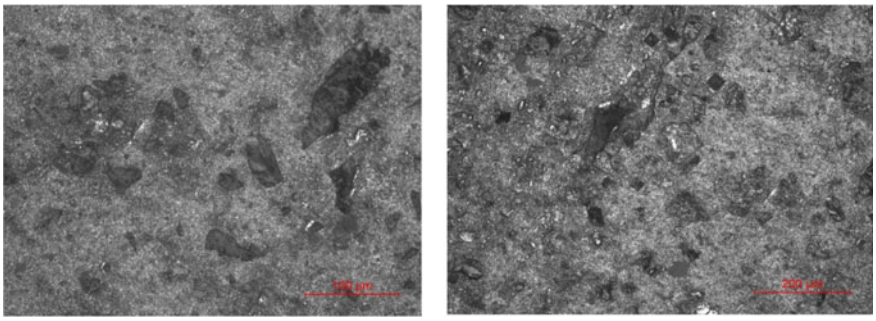

Fig. 3 Surface morphology of crystalline silica-aluminium metal-based hybrid composite

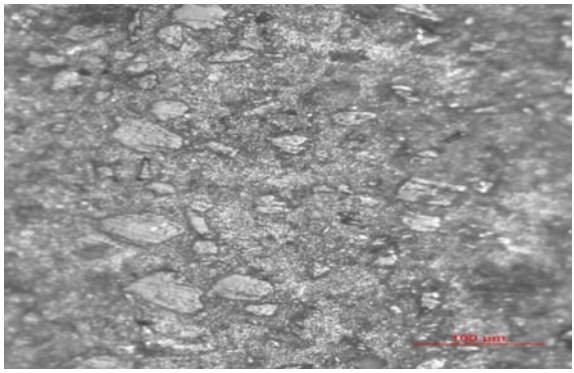

Fig. 4 Crystalline silica-aluminium metal-based hybrid composite after in vitro corrosion test

Figure 4 shows the surface after corrosion test. Crystalline silica-aluminium meta-based hybrid composite makes constructive reaction towards formation of apatite layer under this test environment.

3.3 Bactericidal Study

Antimicrobial activities of candidate metal matrix composite have tested with the degree of growth inhibition of microorganisms and corresponding petri dish has shown in Fig. 5. No zone of inhibition has found out during 24 h of observations as seen in Fig. 5.

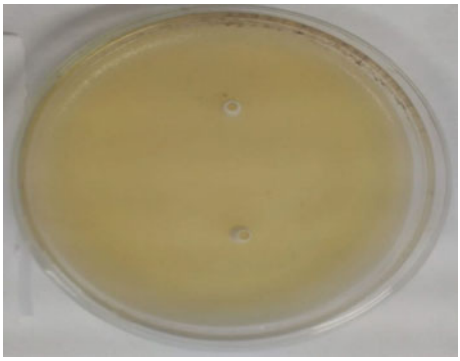

Fig. 5 Bactericidal analysis on crystalline silica-aluminium metal-based hybrid composite

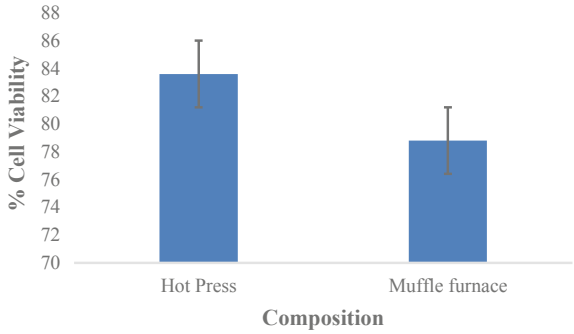

Fig. 6 Cytotoxicity study on crystalline silica-aluminium metal-based hybrid composite

3.4 Cytotoxicity

Figure 6 shows the result obtained from cytotoxicity study based on crystalline silica-aluminium metal-based hybrid composites. Crystalline silica-aluminium metal-based hybrid composite fabricated into hot press shows more than 80% cell viability and hence, considered as non-cytotoxic as per ISO 10993-5 standards.

4 Conclusion

Significant conclusions based on in vitro biocompatibility studies on fabricated crystalline silica-aluminium metal-based hybrid composites are as follows.

- Crystalline silica-aluminium metal-based hybrid composite fabricated into hot press has satisfactory in vitro corrosion properties.
- Crystalline silica-aluminium metal-based hybrid composite possesses practically no bactericidal property. Hence, this fabricated metal matrix composite will not able to kill the existing tissues and not make any adverse reactions to human body after implantation.
- Based on the results on cytotoxicity study, the crystalline silica-aluminium metal-based hybrid composite fabricated into hot press can be classified as non-cytotoxic in nature.

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