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# Beneficial effects of thin film metallic glass coating in reducing adhesion of platelet and cancer cells: Clinical testing

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ABSTRACT

The adhesion of platelet cells is viewed as a first step in thrombus formation, and cancer cell attachment can lead to cancer seeding. In this study, we examined the feasibility of utilizing $Zr_{53}Cu_{33}Al_9Ta_5$ thin film metallic glass (TFMG) to minimize the adhesion of human cancer cells (breast cancer cell, colon cancer cell, and esophageal cancer cell), human and animal platelets. TFMG and pure titanium (Ti) were respectively grown on glass substrates to a thickness of 200 nm using magnetron sputtering. TFMG was shown to reduce surface roughness of approximately 27% than Ti coating did on glass samples. The concentrations of all major ions released from the TFMG were well below toxic levels. TFMG surfaces were more effective than Ti in increasing the contact angle of water, phosphate buffer saline (PBS) and blood from different animal species. The application of TFMG to bare surfaces was shown to reduce the attachment area of human platelets by 77% and that of pig platelets by 63%. TFMG also reduced the attachment of cancer cells by up to ~87%. These characteristics can be attributed to a low surface free energy of TFMG-coated surfaces (31.89 mN/m), which is far below that of Ti-coated (39.25 mN/m) and bare glass (47.80 mN/m). These findings demonstrate the considerable potential of TFMG coatings in the fabrication of medical instruments aimed at preventing the adhesion of platelet and cancer cells.

## 1. Introduction

The surgical instruments used for tumor biopsy, resection, or ablation are prone to cancer cell adhesion when they come into direct contact with tumor tissue. Adhering cancer cells often remain viable and spread to normal tissue, potentially developing into new tumors. Cancer seeding along the needle tract has been reported in patients receiving percutaneous needle biopsy (PNB) for breast cancer and metastatic colorectal cancer [1]. PNB has been found to be associated with 22% of the cases of breast cancer cell seeding [2], and 19% of the cases of metastasis in the liver from colorectal cancer [3]. Cancer cell seeding is associated with endoscopic surgery for cancer resection as well as radio-frequency ablation for tumor destruction. The recurrence of colorectal cancer at the port site of laparoscopic surgery has been reported in 0.8% of cases [4]. Recurrence in surgical wounds can reach 3% in cases where microscopic surgery is performed for neoplasms in the base of the skull [5]. Local tumor destruction by radiofrequency ablation (RFA) is one of the recommended therapeutic modalities for patients with unresectable liver malignancy. However, in 26.7% of patients with primary or secondary liver malignancies, viable cancer cells have been detected adhering to the needles used in ablation therapy [6]. This helps to explain reports of cancer seeding along the needle tract in 12.5% of patients undergoing RFA for primary liver cancer [7]. Researchers have explored a number of strategies, including the surface modification of titanium instruments, to prevent cancer cell adhesion and subsequent tumor seeding [8].

Intravenous cannula systems [e.g. IC needle, central venous catheter (CVC) and PORT-A catheter] are indispensable to fluid resuscitation, nutritional support, and chemotherapy; however, they are prone to the adhesion and aggregation of platelets on the cannula surface. This can lead to thrombus formation and luminal occlusion inside catheters or in vessels [9]. Thus the inhibition of platelet function has been implemented in clinical application to prevent thrombus occlusion of vessels or vascular stents [10]. The PORT-A placement has been found

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associated with thrombus formation with occlusion in 2.5% of patients in a study including 1500 patients [11]. Munck et al. [12] found that 21% of patients with cystic fibrosis suffered from catheter occlusion whereas 4.7% suffered from vascular occlusion. They observed variations in complication rates associated with the different materials used for cannula; for example, the rate of complications associated with polyurethane far exceeded that of silicon. Bourassa et al. [13] noted a positive correlation between surface irregularities and thrombus formation. Using a scanning electron microscope to compare the surface quality of coronary catheters, they determined that the external and internal surfaces of polyurethane (PU) catheters are rougher than those of polyethylene. Similarly, the coating of stainless steel surfaces with $TiO_2$ has been reported as a means to minimize platelet adhesion to biomedical devices [14].

The amorphous structure of metallic glasses (MGs) gives them high strength and smoothness as well as good wear- and corrosion-resistance, far exceeding that of conventional crystalline metals [15]. Bulk metallic glasses (BMGs) exhibit good biocompatibility and biodegradability [16,17]; however, the brittleness of BMGs makes processing difficult, thereby restricting their applicability. Thin film metallic glasses (TFMGs) share the exceptional mechanical properties of BMGs, which makes them a promising coating material for biomedical applications [18–22]. Titanium has been widely used in medical implants for decades [23]; however, the use of TFMG in medical devices is still being investigated. In a previous study, we reported that Zr-based TFMGs exhibit excellent hydrophobicity and resistance to bacterial colonization [22], as well as relatively low surface free energy and coefficient of friction [24]. Previous studies have reported that cellular adhesion strength can be attributed to the surface topography, surface free energy, and chemical composition of the substrate [25–29]. Thus, we directed our efforts at the effects of TFMG on altering the surface properties of glass, with the aim of reducing the adhesion of cancer cells and platelets. We selected an inert glass substrate in order to focus specifically on the effects of TFMG. In the future, we will conduct similar into the use of biomedical materials, such as Ti6Al4V and stainless steel, as substrates in comparison studies. In this study, we selected a Zr-based $Zr_{53}Cu_{33}Al_9Ta_5$ metallic glass system due to its good glass forming ability (i.e. wide composition range and ease in forming an amorphous structure) [30]. The constituent Ta has good biocompatibility, and in a previous study we demonstrated that $Zr_{53}Cu_{33}Al_9Ta_5$ TFMG also possesses good antibacterial properties against *E. coli* and *S. aureus* [22].

## 2. Materials and methods

### 2.1. Experimental procedure & sample preparations

Glass substrates used in this work (D 263™ M, 12 mm in diameter), which can be used as glass coverslips and also used to culture cells on their surface, were from Marienfeld GmbH & Co. KG, Germany. The glass samples were coated with a 200 nm-thick TFMG, which was deposited using radio frequency (RF) magnetron sputtering at a base pressure of $< 1 \times 10^{-7}$ Torr, working pressure of $3 \times 10^{-3}$ Torr in Ar gas, and power of 100 W. For comparison, bare glass substrate samples with a 200 nm-thick Ti thin film were also fabricated using the same deposition parameters. Fig. 1 shows experimental procedure of present study. Bare, TFMG-coated, and Ti-coated glasses were evaluated by means of various experiments, which the details are described in below.

### 2.2. Surface roughness study

The surface roughness of bare and coated glasses was measured using a coherence correlation interferometer (CCI, Taylor Hobson CCI 6000) over a scanning area of 60 μm × 60 μm. Measurements of surface roughness and the adhesion of platelets and cancer cells were performed at five fixed points, indicated by a cross on the glass samples. Each point was located at a distance of 3 mm from the center of the measurement locations. Data are expressed as mean ± standard deviation of eight experiments, each of which included eight replicates.

### 2.3. Contact angle study

Measurements of contact angle were conducted on the Sessile Drop method [31], using 2 μl droplets of de-ionized (DI) water, 10% phosphate buffer saline (PBS), and whole blood and serum from Lanyu pigs, New Zealand white rabbits, and human donors. Serum samples were separated from whole blood samples for use in comparisons. Two commonly used anticoagulants, heparin and ethylenediaminetetraacetic acid (EDTA), were added to the whole blood prior to contact angle measurements in order to prevent coagulation. The drop contact angle was measured using a contact angle goniometer (Sindatec Model 100SB). Images of the droplets were obtained using a digital camera and the contact angle was measured from the tangent to the droplet surface at the point of contact, through the droplet to the solid surface. Data are expressed as the mean ± standard deviation obtained from four experiments.

### 2.4. Surface free energy

Surface free energy (SFE) of bare and coated glass surfaces was calculated using based on the Owens-Wendt equation [32] by measuring contact angles of two fluid samples, DI water (polar) and diiodomethane (non-polar). It should be noted that the contact angle results of blood and serum were not included for calculating the SFE. Owens and Wendt equation is a common and recommended method for evaluating surface free energy (SFE), which has been widely used in many recent articles [33–37]. Despite numerous methods for calculating SFE, such as harmonic mean (HM) and Lifshitz-van der Waals/Acid-Base (LW/AB) method using multi-liquids, the Owens-Wendt method based on the interfacial solid/liquid energy is the suitable approach in this study due to comparable, simple and reliable results [33,35,38]. In addition, the values obtained using Owens-Wendt equation are very close to those of HM and LW/AB methods [33–35,39,40].

### 2.5. Inductively coupled plasma mass spectrometry (ICP-MS)

To investigate the effect of metal ions released from the TFMG, we immersed TFMG-coated samples in saline solution (10 ml, 0.9 wt%) for 30 days. The extracted solution (1 ml) was then examined using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, SCIEX ELAN 5000).

### 2.6. Cell cultures

The human breast cancer cell line MBA-MB-231 and colon cancer cell line WiDr were purchased from ATCC® and the esophageal cancer cell line SK-GT-4 was provided by Dr. Wen-Chien Huang (Department of Surgery, Mackay Memorial Hospital). The cells (density of $1 \times 10^5$ cells/well) were cultured on glass samples at the bottom of 24-well plates. Prior to culturing, the glass samples were sterilized by ethylene oxide (3MSteri-Vac™, 8XL Gas Sterilizer/Aerators, 3M Health Care, MN, USA). The glass samples were immersed in PBS for 1 h before use. The cancer cells were then maintained in growth medium comprising high-glucose (4.5 g/l) Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% fetal bovine serum (FBS), 1% sodium pyruvate, non-essential amino acid, L-glutamine, and phenyl red (alkali). The samples were kept in an incubator at 37 °C in a humidified environment under 5% $CO_2$ for 24 h.

### 2.7. Blood samples

The collection and preparation of blood samples from human