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Titanium wire implants with nanotube arrays: A study model for localized cancer treatment

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ABSTRACT

Adverse complications associated with systemic administration of anti-cancer drugs are a major problem in cancer therapy in current clinical practice. To increase effectiveness and reduce side effects, localized drug delivery to tumour sites requiring therapy is essential. Direct delivery of potent anti-cancer drugs locally to the cancer site based on nanotechnology has been recognised as a promising alternative approach. Previously, we reported the design and fabrication of nano-engineered 3D titanium wire based implants with titania (TiO_2) nanotube arrays (Ti-TNTs) for applications such as bone integration by using *in-vitro* culture systems. The aim of present study is to demonstrate the feasibility of using such Ti-TNTs loaded with anti-cancer agent for localized cancer therapy using pre-clinical cancer models and to test local drug delivery efficiency and anti-tumour efficacy within the tumour environment. TNF-related apoptosis-inducing ligand (TRAIL) which has proven anti-cancer properties was selected as the model drug for therapeutic delivery by Ti-TNTs. Our *in-vitro* 2D and 3D cell culture studies demonstrated a significant decrease in breast cancer cell viability upon incubation with TRAIL loaded Ti-TNT implants (TRAIL-TNTs). Subcutaneous tumour xenografts were established to test TRAIL-TNTs implant performance in the tumour environment by monitoring the changes in tumour burden over a selected time course. TRAIL-TNTs showed a significant regression in tumour burden within the first three days of implant insertion at the tumour site. Based on current experimental findings these Ti-TNTs wire implants have shown promising capacity to load and deliver anti-cancer agents maintaining their efficacy for cancer treatment.

1. Introduction

Current cancer treatments for solid tumours are based on surgical resections followed by chemotherapy or radiation therapy. However, these methodologies pose limitations in cases where patients are unable to undergo surgical resections due to proximity of tumour to a vital organ, size of the tumour and co-existence of prevailing health conditions [1–3]. Overall, in cases with tumours developed in pancreas, stomach, prostate, lung and liver the survival rate of patients is often very low even after surgical resection [1,3–7]. Intravenous drug delivery is also not effective in such cases as the concentration of the drug reaching the tumour site is only a fraction of the actual dose [8]. Furthermore, the remaining drug is distributed throughout the body to healthy tissue and vital organs resulting in severe side effects [9,10]. Many chemotherapeutic drugs also suffer with fast plasma clearance or short half-lives which in turn results in frequent and prolonged administration causing either drug resistance or other adverse effects [11]. In recent years, a new hypothesis has evolved testing out nominally non-invasive localized drug delivery systems to improve survival outcomes in such cases [12,13]. Over the years, many strategies for localized tumour regression or destruction have evolved including cryosurgery [14,15], thermal heating [16–18] and chemical ablation [19]. Such methods have proven beneficial in cases where patients cannot survive harsh surgical operations due to poor health. However, the localized destruction of tumours employing these techniques does cause damage to the surrounding normal healthy tissue. Research attempts are currently being focused on localized delivery of chemotherapeutics directly to the site of tumour to

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increase tumour exposure to high concentration of drugs while limiting systemic toxicity [20–22]. Direct injections to the tumours in accessible areas such as liver [23], lung [24] and pancreas [25] have shown great promise in treatment; however these intra-tumour injections suffer from unbalanced drug distribution at the tumour site and fast clearance rates in the body. Hence, some reports have also proposed using microspheres [26–28] and gels [29,30] which can be injected at tumour site resulting in the formation of drug deposited compartments within the tumour for slow release. Another fast developing approach for localized drug delivery is the development of drug releasing implants that can be inserted directly in or near the tumour site to cause initial tumour regression followed by minimal systemic administration of the anti-cancer drug if required [31–34]. Implantable devices releasing either chemotherapeutic drugs or other anti-cancer agents have been extensively studied and employed in cases of brain tumour where bypassing blood brain barrier is one of the major issues. However, the only clinically approved implant so far in this context is the Gliadel® wafer which is designed to treat glioblastoma multiforme, a severe brain cancer which has a very low patient survival rate [35–37]. These implants maintain drug release over a period of 5 days and have shown to have increased patient survival span [35,38]. Regardless of the success of Gliadel® wafer the use of localized implants has yet to become prevalent in cancer therapy. Two major limitations of Gliadel® wafers is their size (2 cm diameter) which requires considerable surgical intervention and the use of only one specific anticancer drug.

To develop a functional and usable intra-tumour implant for localized drug delivery, it should possess properties such as being able to provide optimal drug concentrations within the tumour site with minimal surgical intervention, should be versatile in carrying a range of therapeutic agents and anticancer drugs or combinations and should be able to maintain effective drug delivery for an extended time period (at least more than 24 h) in order to overcome the shortcomings of daily administration of drug [31,39,40]. Any practically workable implant should be able to release drug in effective concentrations and over a greater tumour distance. Herein, we report the design and fabrication of titanium wire implants engineered with titania (TiO_2) nanotube arrays (TNTs), loaded with potent anti-cancer for localized cancer therapy. Titanium was selected as a metal of choice to fabricate these nano-arrays primarily due to its impressive mechanical and biocompatible properties. The fabrication process of nanotubes by electrochemical anodisation is simple, cost-effective and scalable. Titanium reacts naturally with atmospheric oxygen to produce a passive oxide layer on the outside surface, which makes it a great choice for clinical devices including prosthesis [41], tissue grafts [42,43], dental [44,45] and craniofacial implants [46]. In our previous work, we have verified using several *in-vitro* studies, the potential applicability of TNTs for loading and release of various drugs using planar and wire implants [47–49]. Our group has also pioneered a new method for *ex-vivo* study of drug release employing implantable TNTs wires loaded with drug in bones using the Zetos bone bioreactor [50,51]. A series of drug concentration profiles in bone were obtained in real time maintaining the 3D matrix relationship between the bone cells and the marrow cells and exercise control over the physiochemical bone environment [52].

Ti based nano-array systems have established inherent properties such as high surface area, controllable nanotube dimensions, tuneable geometries and surface chemistry, high and versatile drug-loading capacity for several drugs, ability to modulate drug release kinetics and so forth. Also, the system is generic such that different types of drugs, proteins or growth factors (including their mixtures) could be loaded, thereby providing the ability to design Ti-TNT wire implants with multiple drug release and complex therapies [53]. In addition to above mentioned properties the 3D nature and wire like geometry of the implant offers a handle to insert the implant directly at tumour site without any surgical procedures [52]. While our previous *ex-vivo* studies aided in bridging the gap in our understanding between *in-vitro* and *in-vivo* conditions, the real test of these 3D TNTs wires would be an *in-vivo* investigation employing a cancer model to evaluate the anticancer efficacy of these implants. We designed the current study with the hypothesis to characterize these Ti-TNTs wires as a model for localized drug delivery of the anti-cancer agent TRAIL at the tumour site by direct insertion of the wires subcutaneously directly into already established tumour xenografts, without any major surgical procedures (Scheme 1). Prior to testing TNTs drug releasing efficiency *in-vivo*, we established a cell viability profile in 2D/3D *in-vitro* and *ex-vivo* culture systems using MDA-MB231-TXSA breast cancer cells.

2. Materials and methods

2.1. Reagents

Titanium wire (99.7%) with diameter of 0.5 mm was purchased from Alfa Aesar (USA). Ethylene glycol, ammonium fluoride [NH_4F], 4′,6-diamidino-2-phenylindole ($C_{16}H_{15}N_5$ – DAPI), phalloidin ($C_{35}H_{48}N_8O_{11}S$), crystal violet ($C_{25}N_3H_{30}Cl$) and non-tagged homotrimeric Apo2L/TRAIL were a kind gift from Dr. Avi Ashkenazi (Genentech, Inc., USA). Affinity Pure Goat Anti-Human IgG Fcγ Fragment was purchased from Jackson Immunoresearch Laboratories, Inc., Dulbecco's modified eagle's medium (DMEM), fetal calf serum (FCS), penicillin/streptomycin, and glutamine were purchased from Biosciences (Australia). Trypsin (Gibco); Matrigel HC (BD Biosciences); isoflurane (Faulding Pharmaceuticals); phosphate buffer solution (PBS) (HyClone Laboratories, Inc); Culture flasks; 96-well plate; 8-well chamber slides and 6-well plate (Greiner Bio-One); 10 *v*% formalin solution (Ajax Finechem); Alamar Blue® (Life Technologies Corporation); and Ac-DEVD-AFC (fluorogenic caspase-3(CPP32) substrate) was purchased from Kamiya Biomedical Company; Ultrapure water Option Q–Purelabs (Australia) was used for preparation of all the solutions used in this study.

2.2. Fabrication of nanotube arrays on Ti wire (TNTs)

Commercial Ti wires were cut into lengths of 2.5 cm, were annealed in air for 2 h at 500 °C, followed by sonication in acetone and ethanol. About 8 mm length of each wire was electro-polished at 25 V using perchloric acid electrolyte containing butanol and ethanol, in the ratio P:B:E = 1:6:9 (maintained at 4 °C); and followed by sonication in acetone for 1 h to further remove any impurity. Special electrochemical setup was used for anodising the electropolished Ti wires which only allowed a length of 3 mm to be immersed in the ethylene glycol electrolyte (containing 0.3% w/v ammonium fluoride and 1% v/v water). The anodisation was performed for 20 min at 75 V using computer controlled power supply (National Instruments, USA) [47,50]. Post anodisation Ti-TNTs were rinsed in deionised water, followed by drying in air. Using special wire cutter each Ti-TNTs was cut at 5 mm total length (with active length of TNTs = 3 mm) leaving 2 mm for mechanical handling. All implants were sterilised using ethanol prior to cell studies.

2.3. Structural characterization of TNTs wire implants

The structural characterization of the prepared Ti-TNTs, before drug loading and after drug release was performed using a field