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# The use of collagen-based scaffolds to simulate prostate cancer bone metastases with potential for evaluating delivery of nanoparticulate gene therapeutics

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ABSTRACT

Prostate cancer bone metastases are a leading cause of cancer-related death in men with current treatments offering only marginally improved rates of survival. Advances in the understanding of the genetic basis of prostate cancer provide the opportunity to develop gene-based medicines capable of treating metastatic disease. The aim of this work was to establish a 3D cell culture model of prostate cancer bone metastasis using collagen-based scaffolds, to characterise this model, and to assess the potential of the model to evaluate delivery of gene therapeutics designed to target bone metastases. Two prostate cancer cell lines (PC3 and LNCaP) were cultured in 2D standard culture and compared to 3D cell growth on three different collagen-based scaffolds (collagen and composites of collagen containing either glycosaminoglycan or nanohydroxyapatite). The 3D model was characterised for cell proliferation, viability and for matrix metalloproteinase (MMP) enzyme and Prostate Specific Antigen (PSA) secretion. Chemosensitivity to docetaxel treatment was assessed in 2D in comparison to 3D. Nanoparticles (NPs) containing siRNA formulated using a modified cyclodextrin were delivered to the cells on the scaffolds and gene silencing was quantified. Both prostate cancer cell lines actively infiltrated and proliferated on the scaffolds. Cell culture in 3D resulted in reduced levels of MMP1 and MMP9 secretion in PC3 cells. In contrast, LNCaP cells grown in 3D secreted elevated levels of PSA, particularly on the scaffold composed of collagen and glycosaminoglycans. Both cell lines grown in 3D displayed increased resistance to docetaxel treatment. The cyclodextrin.siRNA nanoparticles achieved cellular uptake and knocked down the endogenous GAPDH gene in the 3D model. In conclusion, development of a novel 3D cell culture model of prostate cancer bone metastasis has been initiated resulting, for the first time, in the successful delivery of gene therapeutics in a 3D *in vitro* model. Further enhancement of this model will help elucidate the pathogenesis of prostate cancer and also accelerate the design of effective therapies which can penetrate into the bone microenvironment for prostate cancer therapy.



## 1. Introduction

Prostate cancer is one of the leading causes of cancer-related death in men and is estimated to cause almost 27,540 deaths this year alone in the United States [1]. The major hurdle to improve the survival rates in prostate cancer arise from difficulties in treating metastatic disease [2]. The bony skeleton is the most common site of prostate cancer metastases (typically the vertebral column, pelvis, ribs, long bones, and skull) and is notoriously difficult to treat [3]. Such metastases are characterised by abnormal bone remodelling demonstrating bone osteolysis and the replacement of lamellar bone with woven bone [4]. Bone cancer metastases are typically treated using chemotherapeutic agents such as docetaxel

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and mitoxantrone [5]. Although these agents have been shown to prolong the length of survival following diagnosis [6–9] they do so only marginally, offering a few months of increased lifespan. Another major limitation is the eventual development of resistance to these chemotherapeutics, which limits the effectiveness of the therapy [10,11]. Furthermore, inadequacies in the treatment of metastatic prostate cancer arise from the relatively poor understanding of the progression of prostate cancer disease, resulting in an unmet clinical need [12].

Bone cancer itself results in great morbidity and reduced patient quality of life arising from pain, fractures and spinal cord and nerve root compression [13]. *In vivo* animal models are useful for disease investigation and can offer some insight into prostate cancer bone metastases development [14]. However, it is recognised that the disease pathology and progression from primary to metastatic cancer in humans may not be accurately reflected by animal models [14–16]. In addition, the use of animal models for such studies are often limited due to lack of availability or indeed ethical issues related to the use of animals in research [16]. Therefore a suitable *in vitro* model of the disease is highly desirable to avoid the use of expensive animal models and to provide results that could be directly related and applied to human disease.

In 2010 it was reported that 70–80% of *in vitro* cancer studies were still conducted using two-dimensional (2D) techniques such as petri dishes and cover slips [17]. This is despite the fact that cancer cell growth *in vivo* differs greatly from these 2D cell culture models and has demonstrated marked differences in terms of cell proliferation rate and gene expression [17]. In the native tissue, cancer cells are surrounded by a three-dimensional (3D) microenvironment which provides biological and physical support whilst modulating cell behaviour, a feature that is lacking in 2D cell culture and results in disparity between *in vitro* and *in vivo* results [15]. Thus, there exists a clear necessity to develop a physiologically-relevant 3D cell culture system both as potential systems for drug development and to enhance our understanding of the disease process and improve the correlation between results obtained in the laboratory and *in vivo* animal and human subjects [18,19].

Collagen I is the primary organic constituent of bone, comprising approximately 95% of the organic matrix whilst the mineral matrix is mostly made up of calcium and phosphate ions in the form of nanohydroxyapatite crystals [20]. 3D collagen-based scaffolds can be engineered to be biocompatible, biodegradable and non-toxic with a pore structure that facilitates the infiltration of cells and nutrients. They have been widely investigated for tissue engineering applications [21] and such scaffolds have been specifically optimised for bone repair [22–25], focusing on the restoration of form and function of tissue insufficiencies [26]. In addition, the use of such scaffolds for the *in vitro* culture of cells in a 3D environment is now recognised to offer potential to enhance our understanding of disease mechanisms and pathology with a resulting improvement in treatment outcomes [15,17,27,28].

In this study, we aimed to investigate metastatic prostate cancer cell culture on different collagen-based scaffolds in order to develop a physiologically relevant 3D bone metastases model which could be used to facilitate investigation of the mechanisms of prostate cancer progression and formation of metastases. Three scaffolds with different compositions were utilised. The first was composed of collagen I and glycosaminoglycan (GAG). GAGs are negatively charged carbohydrates which are common constituents of the extracellular matrix involved in cell adhesion, migration, proliferation and differentiation [21]. This scaffold has been previously optimised for bone tissue engineering applications [21] and has also been investigated for *in vitro* breast cancer cell culture, where it was shown to promote cell growth and infiltration whilst also facilitating osteogenesis [29]. Thus, this scaffold was investigated for its utility as a prostate cancer cell culture platform. Two other scaffolds composed of collagen I with different levels of nanohydroxyapatite (nHA) were also investigated. nHA is known to be both biocompatible and non-toxic [30,31] with a composition similar to the inorganic component of human bone [32]. In addition, the presence of nHA has been shown to improve both the osteoconductive and osteoinductive nature of scaffolds [30]. Scaffolds contained either two-fold nHA relative to collagen (S200 scaffold) or five-fold nHA relative to collagen (S500 scaffold). Because of its presence in bone, it is anticipated that the presence of nHA in collagen-based scaffolds would better mimic the *in vivo* bone microenvironment and would subsequently impact the invasiveness of cancer cells.

Having characterised the 3D model, the scaffolds were then used to evaluate siRNA nanoparticulate delivery. siRNA is a form of RNA interference (RNAi), a method of gene regulation that can silence the expression of target genes, which can be exploited for therapeutic benefit [12]. *In vivo* data previously published using RNAi in the treatment of prostate cancer, in particular using targeted cyclodextrin-based nanoparticles, has been encouraging [33,34]. In addition the collagen-based scaffolds described above have also been used for delivery of other therapeutic nucleic acids previously [30]. Physiologically relevant *in vitro* models of bone metastatic lesions will help to optimise the design of these gene therapeutics and accelerate translation to the clinic.

## 2. Materials and methods

### 2.1. Materials

All items were purchased from Sigma unless otherwise stated. Penicillin-Streptomycin (Gibco) was purchased from Biosciences (Dublin, Ireland). The cationic cyclodextrin was synthesized as previously reported [35]. Anti-GAPDH siRNA (sense strand sequence 5′-CUG CCA AAU AUG AUG ACA U-3′) was purchased from Sigma (Wicklow, Ireland). Negative control non-silencing siRNA (sense strand sequence 5′-UUC UCC GAA CGU GUC ACG UdT dT-3′) was purchased from Qiagen (Manchester, U.K.).

### 2.2. Scaffold fabrication

Collagen-glycosaminoglycan and composite collagen-nHA scaffolds (S200 and S500) investigated in this study were manufactured using a technique recently developed [25,36]. Briefly, collagen slurry (0.5% (w/v)) was fabricated by blending fibrillar collagen (Integra Life Sciences, Inc.) with 0.05 M acetic acid. For the composite scaffolds, nHA particles were synthesized as previously described [30,37] and added to the collagen slurry during the blending process. Two concentrations of nHA suspensions were added (relative to the weight of collagen used), 7.2 g nHA yielding a 2:1 ratio of nHA:collagen scaffold (S200), and 18 g nHA yielding a 5:1 ratio of nHA:collagen scaffold (S500). Slurries were freeze-dried as previously described and all scaffolds were cross-linked and sterilized using a dehydrothermal (DHT) treatment [38]. Cylindrical scaffold samples (8 mm diameter, 4 mm height) obtained using a biopsy punch were further cross-linked with 14 mM N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride and 5.5 mM N-Hydroxysuccinimide (EDAC/NHS) in $dH_2O$ [24] to improve the mechanical properties of the constructs.

### 2.3. Cell culture

PC3 cells (human prostate cancer cell line, European Collection of Cell Cultures (ECACC), UK) were maintained in complete growth media consisting of RPMI-1640 medium, supplemented with 10%