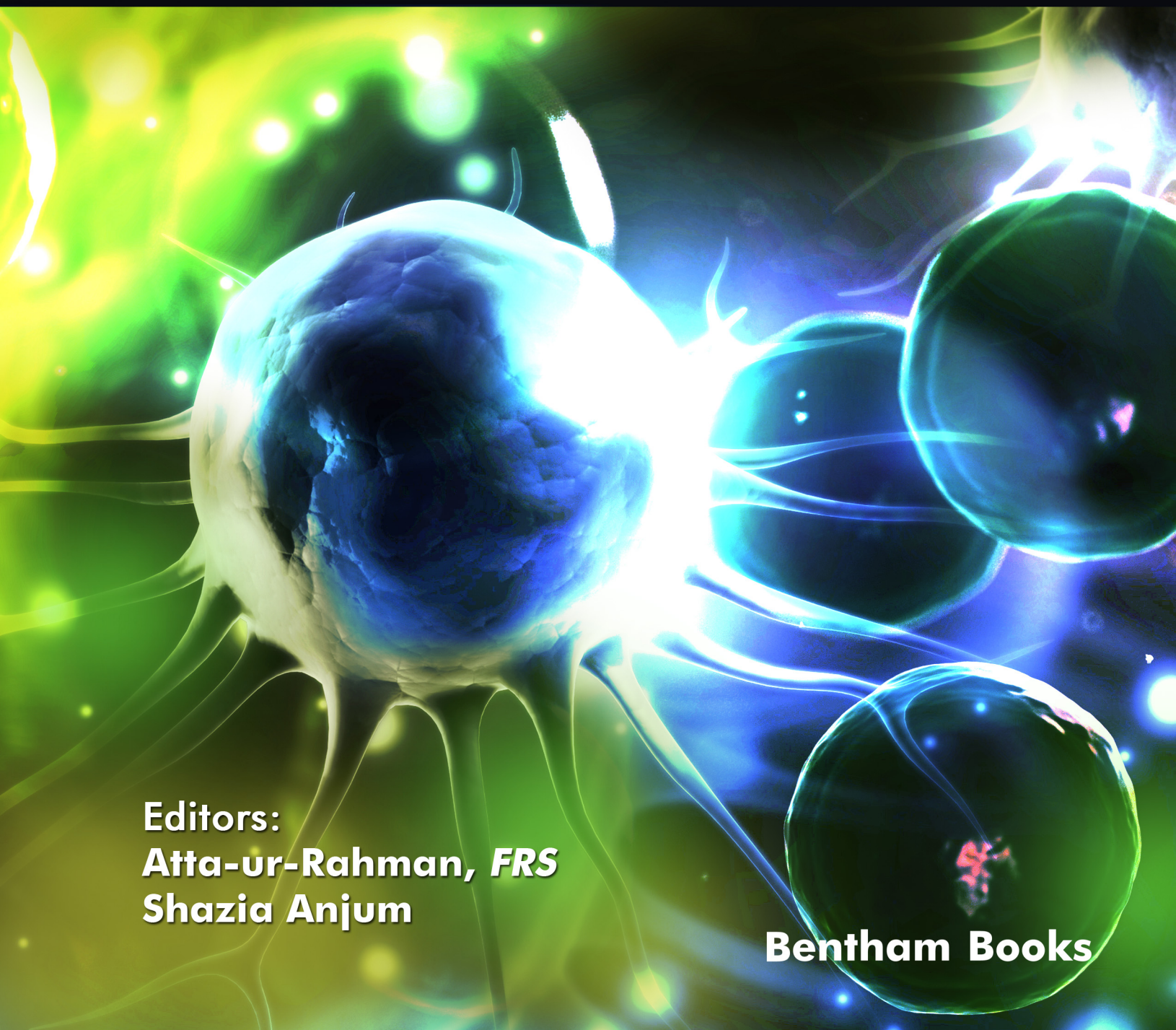
# Frontiers in Stem Cell and Regenerative Medicine Research



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**Shazia Anjum**

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# Frontiers in Stem Cell and Regenerative Medicine Research

# (*Volume 10*)

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## Frontiers in Stem Cell and Regenerative Medicine Research

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# PREFACE

The tenth volume of 'Frontiers in Stem Cell and Regenerative Medicine Research' presents important recent developments in this fast-growing field.

Edda *et al*. in their chapter focus on the differentiation and signaling pathways of osteoblasts and osteoclasts. Pillai *et al*. discuss the role of cancer stem cells (CSCs) in therapy resistance with detailed molecular mechanisms. Ponnuraj *et al*. in the third chapter of the book present the potential use of dental stem cells, particularly stem cells from human exfoliated deciduous teeth (SHED) for tissue regeneration. Masoud *et al*. give an overview of toxicological studies from animal models to stem cell-based methods. In the last chapter of the book, Feng *et al*. discuss the possible mechanisms proposed to explain how certain factors affect the differentiation of MSCs.

We owe our special thanks to all the contributors for their valuable contribution in bringing together the tenth volume of this book series. We are thankful to the efficient team of Bentham Science Publishers for the timely efforts made by the editorial personnel, especially Mr. Mahmood Alam (Editorial Director), Mr. Obaid Sadiq (in-charge Books Department) and Ms. Asma Ahmed (Manager Publications).

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# CHAPTER 1

# Novel Drugs and Their Stem Cell-based Targets for Osteoporosis: Challenges and Proceedings

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**Abstract:** The aging of the population goes along with age-related diseases, such as osteoporosis, a disorder of bone remodeling. Bone homeostasis is maintained by bone-building osteoblasts and bone-resorbing osteoclasts. During osteoporosis, this balance is disturbed by augmented bone resorption, which leads to an increased risk of bone fractures, with potentially lethal consequences. To battle this, various drugs with different target sites are used. Currently, the gold standard osteoporosis medications are the bisphosphonates, which induce apoptosis of the osteoclasts. However, bisphosphonates may cause adverse effects, such as osteonecrosis of the jawbone. Other available drugs for bone metabolism disorders also exhibit undesired side- and off-target effects of varying severity. Thus, new potential drug candidates are being developed, some already reached phase II or phase III clinical trials. The modes of action of these drug candidates range from anti-resorptive to osteoanabolic therapies. Osteoanabolic therapies stimulate the formation of bone, while anti-resorptive therapies decrease the bone resorption. Most anti-resorptive therapies induce apoptosis of the osteoclasts, which negatively affects the osteoblasts as well since there is a feedback loop between these two cell types. A better understanding of bone homeostasis, beginning with the differentiation pathways of mesenchymal stem cells towards osteoblasts and hematopoietic stem cells towards osteoclasts and their interactions during these differentiation processes are of increasing interest for future osteoporosis treatments with minimal side effects. This chapter focuses on the differentiation and signaling pathways of osteoblasts and osteoclasts. In addition, new osteoporosis drugs are illuminated from the biological and the chemical point of view. Their progress from bench to bedside is presented.

**Keywords:** Antiresorptive, Bisphosphonates, Cathepsin K, Hematopoietic stem cells, Osteoporosis, Osteoanabolic, Regenerative medicine, Mesenchymal stem cells, X-ray.

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## OSTEOPOROSIS AND ITS' CHALLENGES

Osteoporosis is a mainly age-related disease, characterized by a dysregulation of bone resorption and formation, which increases the risk of fractures. The World Health Organization (WHO) classifies osteoporosis in the ten most often occurring diseases worldwide, that affected about 200 million people and caused nearly nine million fractures in 2000 [1]. The risk of suffering a fracture of the wrist, hip, or vertebra within the lifetime is about 30-50% for women and 15-30% for men in developed countries [2]. In 2017, the costs for the treatment of osteoporotic patients were estimated at 37.5 billion € in EU [3] and 22 billion $, in 2018, in the USA [4] and are expected to increase.

The major setback in osteoporosis management is its silent nature with no obvious symptoms during early phases of the disease progression, which makes it difficult to diagnose before the first fracture occurs. A closer look at the healthcare cost distribution in the EU, where only 5% is spent on prevention and 95% on fracture repair and long-term treatment, confirms the severity of the problem. Moreover, the International Osteoporosis Foundation estimated that only 25% of all osteoporosis cases are reported [3]. One possible way to improve early osteoporosis diagnosis is to implement the screening of the bone mass by means of dual-X-ray absorptiometry in the risk groups such as post-menopausal women and the elderly. The bone mass of a patient with osteoporosis is equal or less than -2.5 standard deviations of the average bone mass of young and healthy adults between the age of 20 and 29 [5]. Alterations in the bone mass are also indicative of other bone remodeling disorders.

Osteoporosis can be divided into primary and secondary osteoporosis. Both types are not curable nowadays and the only available therapeutic approach is to slow down the loss of the bone mass. Primary osteoporosis is defined by no direct or singular known cause to the disease [6] and is further classified as the idiopathic juvenile osteoporosis, which affects children; postmenopausal and senile osteoporosis, that occur mainly in elderly people. The latter case is associated with the loss of estrogens and androgens, among other contributing factors [7]. These hormonal changes alter several processes within the body and lead to a decreased defense against oxidative stress (OS).

In order to protect cells against OS, mitochondria activate the expression of members from the transcription factor sub-class FoxO. For example, FoxO3 was proven to have a positive effect on osteoblast survival during OS [8]. In addition, the FoxO transcription factors bind β-catenin, which is a co-activator of FoxO transcription, thus enhancing the process in a fast-forward reaction [9]. Furthermore, it is an important transcription factor in the differentiation of

multipotent mesenchymal stem cells (MSCs) towards osteoblasts [10]. This results in a competition between osteoblast survival and the generation of new osteoblasts under OS. Hence, the early phase of postmenopausal osteoporosis is marked by a loss of calcium of up to 200 mg/day in the first 3-4 years, which decreases to 45 mg/day after 5-10 years of osteoporosis [11].

Reviews by Fitzpatrick or Brown outline that secondary osteoporosis can occur due to nutritional or lifestyle factors, inflammatory causes, genetic disorders, or be induced by medical treatments [6, 12]. The relationship between prolonged or continuous medical treatments with proton pump inhibitors, selective serotonin receptor inhibitors, and other medications and secondary osteoporosis have been reviewed by Panday and colleagues [13]. Another class of drugs associated with secondary osteoporosis is the glucocorticoids and other corticosteroids. These drugs are used to suppress inflammations during chemotherapy, asthma, or allergic reactions. Notably, glucocorticoids can regulate the differentiation of MSCs towards osteoblasts under normal circumstances, but can also cause apoptosis of osteoblasts by inducing OS [14, 15]. When applied in high concentrations, glucocorticoids increase adipogenic differentiation to the disadvantage of osteogenic differentiation [16]. This effect is also mediated by an inhibition of Wnt signaling by the upregulation of Dickkopf-1 (DKK-1) [17].

## FROM OSTEOGENIC LINEAGES TO BONE FORMATION AND RESORPTION

The Wnt signaling, which is negatively affected by glucocorticoids during secondary osteoporosis, is thought to be a key pathway of osteogenesis. In the following section, the significance of Wnt, BMP, Notch, and Hedgehog signaling pathways in osteogenesis, as well as the differentiation of hematopoietic stem cells (HSCs) towards osteoclasts (osteoclastogenesis), is presented.

### Development of Osteoblasts from Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are of high interest for tissues and organ bioengineering approaches due to their accessibility and broad differentiation potential, including the osteogenic lineage [18 - 20]. According to the International Society for Cellular Therapy, MSCs are defined by their adherence to plastic under standard culture condition, the expression of at least three markers CD105, CD90, and CD73 and the lack of expression of several surface molecules, namely CD45, CD34, CD79α or CD19, CD14 or CD11b, and HLA-DR. In addition, the cells must be able to differentiate towards the osteogenic, adipogenic, and chondrogenic lineage *in vitro,* as demonstrated by specific stainings [21]. MSCs can be isolated from various tissues, the major ones being adipose tissue, bone marrow, and umbilical cord. The site of the isolation has a prominent effect on the

# CHAPTER 2

# The Role of Cancer Stem Cells in Disease Progression and Therapy Resistance

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**Abstract:** A small subpopulation of tumour cells, known as cancer stem cells (CSCs), is the main culprit of tumour growth. They are capable of self-renewal, tumour initiation, expansion, metastasis, therapy resistance and cancer relapse. Factors associated with malignant properties of CSCs include decreased apoptotic insults, enhanced activity of drug efflux pumps and capacity to induce DNA repair, expression of detoxification enzymes and ability to become quiescent, *i.e.* phenotypic and genotypic plasticity of CSC, *etc.* These extraordinary capabilities of CSCs contribute to therapeutic resistance and cancer recurrence. Moreover, multiple factors including a complex network of tumour stroma, epidermal microenvironment and different sub-compartments within the tumour stimulate CSCs plasticity-mediated tumour progression. These factors along with the metabolic flexibility of CSCs help them to become more aggressive, subsequently leading to tumour progression. Therefore, in this chapter, we describe how CSCs are associated with the initiation and progression of cancer. We also discuss the role of CSCs in therapy resistance with detailed molecular mechanisms, all of which could help us in developing promising strategies to benefit cancer treatment.

**Keywords:** Cancer initiation, Cancer progression, Cancer stem cells, CSC plasticity, Signalling pathways, Therapy resistance, Tumour microenvironment.

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## INTRODUCTION

Carcinogenesis involves a series of genetic and epigenetic changes of non-neoplastic proliferative cells, which leads to the formation of highly heterogeneous, progressive and malignant cancer cells [1]. Hence, cancer is an extremely heterogeneous disease consisting of cells with a difference in morphology, genetics, the ability of proliferation and invasion [2]. This heterogeneity may result from hierarchically organized cancer cells, namely cancer stem cells (CSCs) [3, 4]. These CSCs have been found to be associated with cancer initiation, progression, therapy resistance and cancer relapse. CSCs could directly become non- tumourigenic by differentiation and non-tumourigenic (differentiated cell state) ones may switch to tumourigenic (CSC) by dedifferentiation process. CSCs, like normal stem cells, have enormous ability to induce self-renewal and multi-lineage differentiation [4, 5]. Also, CSCs are able to regenerate tumours in serial xenotransplantation assays [6, 7]. CSCs produce all cell types in cancer, which subsequently results in cancer heterogeneity [8]. Furthermore, CSCs concept of carcinogenesis implicates a suitable platform to explain how cancer cells acquire therapy resistance [9]. Accumulating research suggests that slow cycling CSCs can easily avoid conventional anti-proliferative chemo-radiotherapies, which contributes to therapeutic failure, thereby leading to disease progression [9, 10]. It has been suggested that conventional therapeutic regimen (s) target only a bulk of tumour cells, where CSCs remain untreated or they escape these therapeutic insults so that they can effectively repopulate the tumour [11]. Multiple factors including over-activation of drug-efflux pumps, increased DNA repair capacity, activation of detoxification enzyme, hyperactivation of growth signalling pathways, generating plastic phenotypes, and activation of quiescent state, can contribute to therapy resistance property of CSCs [12]. In this chapter, we describe the role of CSCs in cancer initiation, angiogenesis, invasion, metastasis, and therapy resistance.

## CANCER STEM CELLS CSCS IN DISEASE PROGRESSION

### Cancer Stem Cells CSCs in Tumour Initiation

CSC theory of carcinogenesis implies that a subset of cells having stem cell properties is responsible for initiation and maintenance of cancer according to the results of studies identifying CSCs in various cancers [13]. The roles of CSCs in cancer initiation and progression are being discovered day by day. Considering their role in driving tumour growth through self-renewal and differentiation capability, it is believed that the normal stem cells/progenitor cells give rise to CSCs in tumour tissues [14, 15]. Normal stem cells usually stay in a quiescent

state until they receive a growth-stimulating signal. A highly regulated signalling network is maintained in normal stem cells [15]. However, dysregulation of signalling network occurs upon various genetic and/or epigenetic alterations, which ultimately leads to uncontrolled proliferation and possible tumourigenesis (Fig. **1**). The CSCs have the capacity to repopulate parental tumour even when they are present in a very low number [16]. On the other hand, the non-CSC differentiated counterpart does not exhibit a similar kind of tumourigenicity [16]. Experimental results using both *in vitro* and *in vivo* models have shown that CSCs can initiate carcinogenesis in various cancers [17, 18]. For example, transcription factor SOX2 plays a key role in the initiation and progression of melanoma in a mouse model of skin carcinogenesis [19]. SOX2 is found to be expressed at an early stage of tumour formation while it appears to be absent in normal skin. Not surprisingly, cells with SOX2 expression were found to propagate tumour after transplantation, while withdrawal of SOX2-positive cells from established tumours results in growth regression [19]. Therefore, SOX2 expression proves to be a vital factor as it contributes to tumour initiation and progression. Another cancer stem cell biomarker such as CD44, especially CD44v isoforms, has been found to play critical roles in tumour initiation and chemoradioresistance of CSCs. [18]. In liver cancer stem cells (LCSCs), CD133 is one of the most commonly expressed cancer biomarkers, and its expression in LCSCs is associated with higher *in vivo* clonogenicity and *in vitro* tumourigenicity than those of the CD133 counterparts [17].

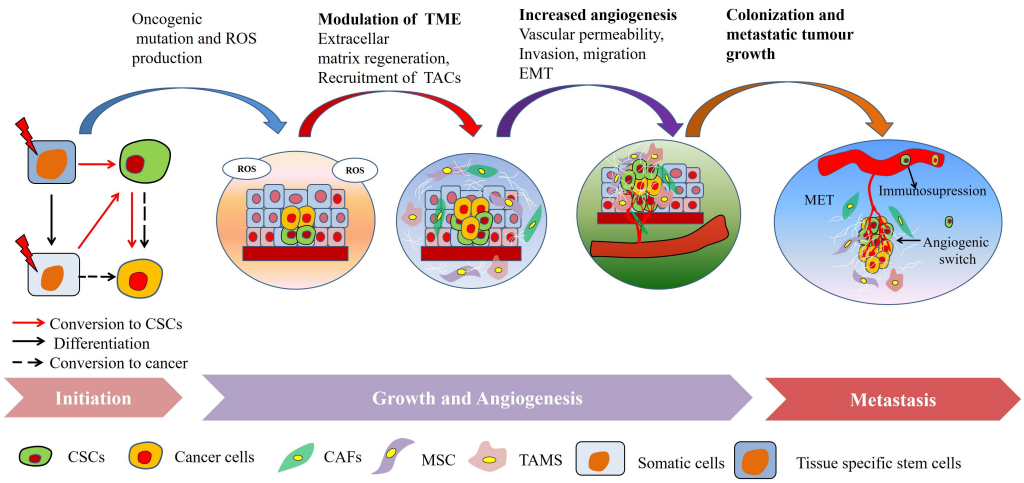


**Fig. (1).** Role of CSCs in carcinogenesis, a key cellular event during tumour initiation, tumour growth, angiogenesis and metastasis.

# CHAPTER 3

# Stem Cells from Human Exfoliated Deciduous Teeth in Tissue Regeneration

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**Abstract:** Every species in this world, from the simplest to complex organisms, has auto-capacity for tissue regeneration. Tissue regeneration is a process of renewal and growth that replaces or repairs damaged or lost tissue as a result of natural changes or disturbances. As organisms become more complex, their regenerative ability diminishes. As humans are complex having very limited regenerative capability, tissue regeneration has become one of growing areas of research. However, it has become a resource-intensive research as it is dependent on the availability and ability of the cells used. The need to find an available source of cells led researchers to choose stem cells. The use of stem cells has shown excellent progress in tissue regeneration and numerous types of stem cells have been reported to be used in tissue regeneration, namely mesenchymal stem cells, neural stem cells, adipose stem cells, cardiac stem cells, induced pluripotent stem cells *etc.* However, the potential use of dental stem cells in regenerative medicine has not been widely discussed. Dental stem cells, which were first discovered in 1985 have been very well-characterized for their potential to be used in dental tissue regeneration, but less recognized for application in other parts of the body. Therefore, this chapter focusses on the potential use of dental stem cells, particularly stem cells from human exfoliated deciduous teeth (SHED) for tissue regeneration. SHED are adult stem cells that can be retrieved from primary teeth. Since these cells can be acquired after extraction of deciduous teeth, they provide non-invasive, unlimited cell sources without any ethical concerns. They are multipotent stem cells with a higher proliferation rate and differentiation capability than other dental stem cells and human bone marrow mesenchymal stem cells. Therefore, this chapter will specifically address the differentiation potential of SHED, particularly with respect to fibroblasts, epithelial and osteoblast-like cells, growth factors and the signalling pathways involved. Knowledge about the differentiation potential of SHED is important because it creates a plethora of opportunities as an excellent stem cell model for tissue regeneration. Keeping this in mind, this chapter aims to provide information to the researchers, students, and scientists working or interested in exploring the SHED on tissue regeneration.

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**Keywords:** Dental stem cells, Differentiation, Epithelial-like cells, Fibroblast-like cells, Mesenchymal stem cells, Odontoblast-like cells, Osteoblast-like cells, Stem cells from human exfoliated deciduous teeth, Tissue regeneration.

## INTRODUCTION

Regeneration of impaired tissues caused by severe injuries, chronic diseases, trauma, infection, and tumour resection is very challenging in clinical practice. As the organisms become more complex, their ability to regenerate ability decreases. Humans, as complex organisms have very limited regeneration capability, but with advances in tissue engineering, tissue regeneration has become a growing area of research. Tissue regeneration is a process of renewal and growth that replaces or repairs damaged or lost tissue due to natural changes or disturbances. It uses tissue engineering as the main approach that involves high cost in the market and is expected to achieve beneficial outcomes. Recent statistics show that the market for tissue engineering stood at around US$9.9 billion in 2019. From 2020 to 2027, it is projected to have a compound annual growth rate (CAGR) of 14.2% [1]. This promising market value of tissue engineering is influenced by relentless cutting-edge needs in regenerative clinical treatments along with the increasing prevalence of many disorders or diseases.

Regeneration of *de novo* tissues requires functional cells and/or growth factors. However, this is a resource-intensive research as it depends on the availability and ability of the cells used. For many years, the use of mesenchymal stem cells (MSCs) has made excellent progress in tissue regeneration. The most common source of MSCs has been bone marrow *via* the painful and invasive aspiration procedure. Nowadays, MSCs can also be obtained from various sources *viz* human embryonic stem cells, placenta, adipose tissue, skin, Wharton's Jelly, umbilical cord blood, human induced pluripotent stem cells, amniotic fluid, as well as the orofacial area. Despite their benefits, some of these cell sources create barriers in applications that force researchers to find new sources. For example, orofacial MSCs, Wharton's Jelly, and adipose tissue require enzymatic treatment [2 - 7]. Meanwhile, MSCs from the placenta and umbilical cord can only be used in allogenic applications [8, 9]. In addition, numerous types of stem cells have also been reported to be used in tissue regeneration, namely cardiac stem cells, neural stem cells, adipose stem cells, induced pluripotent stem cells, *etc.* However, there is dearth of information on the utility of dental stem cells in regenerative medicine. Dental stem cells are a specific type of MSCs [10], that also express specific markers only expressed by MSCs as well as embryonic stem cells, such as *NANOG, CD106, STRO-1*, and *OCT4* [11 - 16]. Dental stem cells, first discovered in 1985 by Yamamura [17] have been well-characterized for their role in regeneration of dental tissues, more precisely in the regeneration of dental

pulp. However, these are less recognized and reported in regenerative medicine applications.

The dental stem cells can be derived from teeth, apical papilla, dental follicle, dental pulp, periodontal ligament, and related dental tissues, thus producing various populations; dental follicle stem cells (DFSCs); dental pulp stem cells (DPSCs); gingiva-derived MSCs (GMSCs); oral epithelial progenitor/stem cells (OESCs); periodontal ligament stem cells (PDLSCs); periosteum-derived stem cells (PSCs); salivary gland-derived stem cells (SGSCs); tooth germ progenitor cells (TGPCs); stem cells from the apical papilla (SCAP); and stem cells from human exfoliated deciduous teeth (SHED) [18].

The dental stem cells show more advantages compared to other stem cells due to their ability to transform not only into specific dental cells, but also into chondroblasts, osteoblasts, neurons, adipocytes, bones, muscle and connective tissues [19, 20]. They are also highly accessible as they could be obtained from dental tissues of young and adult patients. Furthermore, these multipotent stem cells have a higher proliferation capacity than bone marrow mesenchymal stem cells (BMMSCs), are cost-effective, less invasive [20], and have fewer ethical considerations. They also demonstrate similar characteristics as MSCs, particularly having the capability for plastic adherence *in vitro* with the formation of colonies [21], thus indicating the capability of cells in the expansion *in vitro* for a long term.

Of all the dental stem cells mentioned, SHED and DPSCs have been widely used in numerous *in vitro* studies because they have similar characteristics to those of BMMSCs [22]. Moreover, SHED have been known to be an excellent candidate for bone tissue regeneration [23], as reported that these cells, when being injected into the immunodeficient mice, could form the dentin or bone with high capacity [12]. Hence, this chapter focusses on the potential use of SHED as a cell source in tissue regeneration application in relation to its differentiation, potential growth factors involved, as well as possible related signalling pathways.

## INSIGHT INTO STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

SHED are a population of adult stem cells that originate from the embryonic neural crest as ectodermal MSCs [24]. These multipotent stem cells are present in the 6$^{th}$ week of the embryonic stage during human development. Under an inverted microscope, these cells appear to be spindle in shape, flattened, with an elongated body and a large nucleus, morphologically similar to the fibroblasts (Fig. **1**).

# CHAPTER 4

# The Fate of Toxicological Studies: From Animal Models to Stem Cell-based Methods

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**Abstract:** After the development of a new drug, it is compulsory to test its benefits as well as toxic effects before human implementation. In the past, animals were being used as standard models for drug toxicity testing, but animal testing arose many ethical concerns and controversies. To overcome these ethical hurdles, many non-animal toxicity models were developed to cope with the drug toxicity analysis, but certain limitations like interspecies barriers do not make them good models for drug toxicity studies.-. Due to their self-renewal and capacity to divide into multiple cell lineages, such as hepatocytes, cardiomyocytes, and neural cells, stem cells are being used to establish alternative approaches for toxicological studies. This makes them a potential resource in predictive toxicological studies without the limitations of interspecies boundaries. *In-vitro* toxicological models, such as Adult Stem Cells (ASCs), Embryonic Stem Cells (ESCs), and recently established Induced Pluripotent Stem Cells (iPSCs) are currently being used as alternatives to animal models. This chapter will discuss the journey of toxicity studies from animal models to *in vitro* stem cell-based toxicity models.

**Keywords:** Adult Stem Cells, Drug toxicity analysis, Embryonic Stem Cells, Induced Pluripotent Stem Cells, Non-animal methods.

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## INTRODUCTION

Toxicity evaluation is a key component of the drug discovery process and pharmaceutical research. Animals are being used as experimental subjects in drug testing and other health-related studies, which has decreased the incidence of human disease, despite ethical issues for over a century [1]. Toxicological tests for new drugs developed are essential to be tested on animals because they share almost the same molecular pathways as humans [2, 3]. Moreover, if the new drugs are not tested on animal models, it would be unethical to try them on humans directly [4]. That's why millions of animals like mice, rabbits, dogs, and monkeys have been used as drug testing models, but their number has declined recently [5]. In recent times, this thought has been developed that due to differences in anatomy, genetics, and physiology; animal models are not reliable enough to understand and predict human diseases as well as drug toxicity responses [6] like skin irritation, acute toxicity, and reproductive toxicity, *etc* [7]. About 40% of medications may be withheld from clinical testing owing to toxicity that was not observed upstream. Furthermore, animal slaughter raised ethical questions about the suffering and discomfort that these laboratory animals experienced [8 - 10]. Animal research is both costly and time-consuming. Despite the vital role of animals in toxicological studies, the 3Rs (Replacement, Reduction, and Refinement) principle was adopted in 1959 to reduce the usage of animal models and search for alternative *in vitro* toxicity assays [11, 12].

Great efforts have been devoted to improving the stem cell toxicology methods for toxicity testing [13, 14]. *In vitro* cell cultures are of great value because they can provide unlimited cells used for toxicity analysis [15]. Scientists have been operating *in vitro* cell-based assays, which are more similar to *in vivo* studies and provide a better outcome of a toxicological study [16]. *In vitro* based methods have an advantage over conventional toxicity analysis methods because of the well-developed cell culture protocols and the need for a much shorter time as compared to *in vivo* studies [17, 18]. One such *in vitro* model is based on stem cells having the innate ability of self-renewal and have a high potential to differentiate into various cell lineages. These include embryonic stem cells (ESCs), Mesenchymal stem cells (MSCs) [19, 20], and human-induced pluripotent stem cells (iPSCs) [21, 22], which display pluripotent properties thus having the capacity to differentiate into various cell lineages [23 - 25]. iPSCs are used to produce many different cell types by using state-of-the-art stem cell technologies. The genetic makeup, physiology, and pathology of these stem cells are like the organs from which the stem cells are taken. iPSCs maintained in 2D/3D culture systems are unlimited sources of cells. Hepatocytes and beating cardiomyocytes have been successfully produced from pluripotent stem cells and

ESCs, which are used in toxicity studies [26, 27]. Therefore, stem cell-based systems provide a potential approach to obtain cells for drug toxicity analysis, which further leads to the development of better drugs with higher efficiency than animal-tested drugs [28].

Several factors, such as phenotypic characterization and cell selection, culture conditions, experiment size, dosing routine, and selection of possible biological endpoints in the light of human risk, have a significant impact on *in vitro* stem cell assays [29, 30]. The use of iPSCs has its difficulties because they are produced by reprogramming of somatic cells; thus, they are difficult to reprogram. Production of iPSCs is a laborious process, therefore, it requires a lot of time, and often, the number of cells varies from batch to batch. The cost has been reduced partly by using large-scale stirred suspension bioreactors and by microencapsulation of iPSCs with a hydrogel that results in the maintenance of pluripotency of iPSCs [31 - 34]. In this chapter, we look at how different stem cells, such as MSCs, iPSCs, and ESCs, can be used to determine drug toxicity using *in vitro* cultures as an alternative to animal testing.

## Drug Toxicity Models

Recently, a trend has started to use non-animal methods of drug toxicity testing, which eliminates painful procedures while the welfare of animals is prioritized [16]. Many new tests have been developed, like Hen's Egg Test-Chorioallantoic Membrane (HET-CAM), which can be used to assess the irritancy of any eye drug [35]. The Bovine Cornea Opacity/Permeability (BCOP) assay, which can be accomplished by extracting byproducts from dead cows, was being used to determine the toxic effects on the cornea [36, 37]. To monitor the drug's eye irritancy properties, dead rabbit and chicken eyes were used [38, 39]. Human corneal epithelial cells (HCE) and human skin-derived epidermal keratinocytes were used to create 3D epithelial models, which are currently available under the names EpiOcular™ and SkinEthic™, respectively [40, 41].

The neural red uptake (NRU) assay could be used to measure the viability of keratinocytes as an alternative to the animal skin irritation assay [42]. To validate corrosive skin potential, Corrositex™ assay has been used, which replaces painful animal experiments [43]. A 'skin-on-a-chip,' which can assess skin cell viability and skin inflammation, has recently been developed for measuring the toxicity of cosmetics and medications [44]. *In vitro,* toxicological study models used as an alternative to animal models are shown in Table **1**.

# CHAPTER 5

# Effect of Material Properties on Differentiation of Mesenchymal Stem Cells

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**Abstract:** Mesenchymal stem cells (MSCs) have been widely used in the areas of tissue engineering and regenerative medicine due to their wide differentiation potential into various lineages. The stem cell/material interface involved is a complex microenvironment where material can direct the stem cell's fate through its inherent properties (such as stiffness, surface topography and/or surface chemistry, and nanoparticles themselves, *etc.*). Stem cells in contact with materials are able to sense their properties and translate parallel signaling information into stem cell lineage commitment and differentiation. These materials can be utilized as scaffolds for tissue engineering and regenerative medicine and as nanoparticles for drug delivery or cell tracking. Thus, it is of vital importance to investigate the effects of material properties on the differentiation of MSCs to give a better design of biomaterials. With this in mind, we summarize the recent reports about the effects of materials properties (such as stiffness, surface topography and/or surface chemistry, and nanoparticles themselves, *etc.*) on the differentiation of MSCs. We also overview a subset of the possible mechanisms proposed to explain how the material properties affect the differentiation of MSCs.

**Keywords:** Cell/Material Interface , Differentiation , Mesenchymal Stem Cells , Nanoparticles , Regenerative Medicine , Stem Cell Fate , Stiffness , Surface Topography , Surface Chemistry , Tissue Engineering .

## INTRODUCTION

Nowadays, the stem cell-based tissue engineering strategy is a promising technology in clinical applications for damaged/diseased tissue repair [1]. Mesenchymal stem cells (MSCs) are multipotent cells capable of self-renewal and

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multilineage mesenchymal differentiation. Thus they play important roles in the fields of tissue engineering and regenerative medicine [1, 2]. MSCs can differentiate into a variety of cell lineages like osteoblasts, chondrocytes, adipocytes, tenocytes, and neurocytes. Originally identified in the bone marrow, MSCs can also be isolated from various other sources, including adipose tissue, muscles, amniotic fluid, and placenta [3 - 5]. In particular, bone marrow and adipose tissue are two attractive sources for MSCs isolation, and human bone marrow/adipose-derived MSCs have been proven to have great potential for applications in tissue engineering.

A number of signaling pathways and transcription factors regulate the osteogenic and adipogenic lineage commitment and differentiation of MSCs. Several signaling cascades, including Wnt/β-catenin signaling, Hedgehog signaling, and NEL-like protein 1 (NELL-1) signaling play important roles in both adipogenic and osteogenic differentiation [6 - 8]. In terms of transcription factors, runt-related transcription factor 2 (Runx2), the initial and most specific marker, can activate and regulate osteogenesis by increasing the expression of downstream genes [9]. Alkaline phosphatase (ALP) is an early marker for osteogenic differentiation, continuously correlating with the area of high ossification [10]. Osteocalcin (OCN) is a specific marker of mature osteoblasts, which is synthesized only by fully differentiated osteoblasts [11]. Osteopontin (OPN), another marker for osteogenic differentiation, can enhance mineralization [12]. In the case of adipogenic differentiation, peroxisome proliferator-activated receptor gamma (PPARγ) is generally regarded as a master regulator, which can trigger the entire program of adipogenesis [13]. CCAAT/enhancer-binding protein alpha (C/EBPα), another main transcription factor for adipogenesis, can cause a higher sensitivity for insulin and increase the expression of PPARγ [14]. Adiponectin is exclusively expressed in adipocytes and is involved in glucose metabolism [15]. Among them, Runx2 and PPARγ act as the master regulators of osteogenesis and adipogenesis, respectively. The signaling cascades promoting osteogenic and adipogenic differentiation of MSCs generally converge on these two key transcription factors [2].

Many kinds of biomaterials such as polymers, ceramics, and metals are commonly applied in tissue engineering and regenerative therapies, and they are consistently refined with time [16]. In recent years, along with the rapid development of nanotechnology and nanomedicine, nanoparticles (NPs) are playing more and more important roles in biomedical and bioengineering fields. They have great potential for various applications, including drug/gene delivery, bio-imaging, cell labeling, pathologic diagnosis, and disease treatment [17 - 19]. Alternatively, nanoparticles can be immobilized and used in tissue engineering scaffolds or surface coatings on implants [20, 21]. When applying both MSCs and

biomaterials (including NPs) in tissue engineering and/or regenerative medicine, it is of vital importance to investigate the effects of material properties on the differentiation of MSCs to give a better design of biomaterials. The stem cell/material interface involved is a complex microenvironment in which the material can direct the stem cell's fate through its inherent properties (such as stiffness, surface topography and/or surface chemistry, and nanoparticles themselves, *etc.*) [22]. Stem cells in contact with materials are able to sense their properties and translate parallel signaling information into stem cell lineage commitment and differentiation. Recent studies have advanced the hypothesis that the inherent properties of materials can influence, and perhaps even induce, lineage-specific stem cell differentiation by virtue of their inherent stiffness, surface topography and/or surface chemistry, and nanoparticles themselves, *etc* [23 - 28]. The diversity of inherent material properties known to influence stem cell fate represents a tremendous opportunity for stem cell biologists and materials scientists to work collaboratively. With this in mind, we summarize the recent reports about the effects of materials properties (such as stiffness, surface topography and/or surface chemistry, and nanoparticles themselves, *etc.*) on the differentiation of MSCs. We also overview a subset of the possible mechanisms proposed to explain how the material properties affect the differentiation of MSCs.

## MSCS RESPONSE TO STIFFNESS

Cells respond to their complicated microenvironment, which is composed of neighboring cells, extracellular matrix (ECM), as well as autocrine and paracrine soluble growth factors [29]. Stiffness of the cell's environment is relevant to all stages of development, from embryogenesis to terminal cell differentiation [30]. Thus, one of the principles or the design of new biomaterials to control physiological cellular responses using non-biological cues is biomimicry of ECM [31], such as stiffness. As an important mechanical property of biomaterials, stiffness defines the quantity of vital force. It can play key roles in regulating biochemical signaling pathways and thus influence MSC's fate, such as cell adhesion, spreading, proliferation and differentiation.

Hydrogels are usually employed to investigate cell response to stiffness *in vitro* due to their easily tailored mechanical properties by the degree of crosslinking. Hydrogels can be synthesized from an array of polymers (such as poly(ethylene glycol) (PEG), polyacrylamide (PAAm), methacrylated hyaluronic acid (MeHA), silk fibroin, *etc.*) with the desired stiffness varying from several Pa to MPa to mimic natural tissue (from the soft brain to stiff bone). It is believed that mimicking the stiffness of a particular tissue type can guide cellular behavior toward a particular phenotype [29, 32]. Hydrogels can also be consisted of natural

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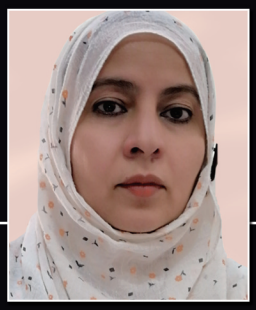
## ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/ Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.



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As recognition of her contributions to science, she has been awarded with 03 International awards like Fellowship from Islamic World Academy of Sciences, Postdoctoral fellowship from Ministry of Culture and Education, Spain and a Young Chemist Award from Third World Academy of Sciences, Italy. She also has several national awards on her credit.