

# **Effect of Mesenchymal Stem Cells Injection on Induced Stomatitis in Chemotherapy Treated Rats**

*Thesis Submitted to*

**Faculty of Dentistry - Ain Shams University**

*In Partial Fulfillment of the Requirements  
of Doctorate Degree in Oral Biology*

**By**

**Asmaa Serry Mohammed**

**B.D.S (2002) faculty of dentistry - Mansoura University**

**Master degree of oral biology (2012)**

**Faculty of oral and dental medicine – Cairo University**

*Assistant Lecturer of Oral Biology*

*Faculty of dentistry Beni-Suef University*

**Supervisors**

**Prof. Dr. Souzi M.Farid Shinaishin**

**Professor of oral biology department**

**Faculty of dentistry- Ain Shams University**

**Dr. khaled El Sayed Nour El Haddad**

**Lecturer of oral biology**

**Faculty of dentistry- Ain Shams University**

**Faculty of Dentistry-Ain Shams University**

**2017**

## List of contents

<b>Title</b>	<b>page</b>
1. Introduction-----	1
2. Review of literature-----	3
2.1. Chemotherapy-----	3
2.1.1 Mechanism of action-----	3
2.1.2. Types of chemotherapy-----	4
2.1.2.1 Alkylating agents-----	4
2.1.2.2. Anti-metabolites-----	5
2.1.2.3. Anti-microtubule agents-----	5
2.1.2.4. Topoisomerase inhibitors-----	6
2.1.2.5. Cytotoxic antibiotics-----	7
2.1.3 Side effects of chemotherapy-----	8
2.2. Oral mucositis:-----	9
2.2.1. Pathogenesis of oral mucositis -----	10
2.2.2. Signs and symptoms of oral mucositis-----	14
2.2.3. Grading of oral mucositis-----	16
2.2.4. Factors influencing oral mucositis-----	18
2.2.5. Treatment of mucositis-----	20
2.2.5.1. Intensive oral care protocol-----	20
2.2.5.2. Antimicrobial agents-----	21
2.2.5.3. Anti-inflammatory agents-----	21
2.2.5.4. Nutritional supplements-----	21
2.2.5.5. Natural and homoeopathic agents-----	22
2.2.5.6. Bio-stimulants-----	22
2.2.5.7. Cryotherapy-----	22
2.2.5.8. Low-energy laser therapy-----	23
2.3. Stem cells-----	23
2.3.1. Properties of stem cells-----	23
2.3.2. Routes of administration of stem cells-----	25
2.3.3. Sources of stem cells-----	26
2.3.3.1. Embryonic stem cells-----	26

2.3.3.2. Adult stem cells-----	27
2.3.3.2.1. Umbilical cord derived stem cells-	27
2.3.3.2.2. Adipose-derived stem cells-----	28
2.3.3.2.3. Cutaneous stem cells-----	28
2.3.3.2.4. Dental stem cells-----	28
2.3.3.2.5. Bone marrow-derived stem cells--	29
2.3.4. Effect of mesenchymal stem cells on wound----	29
Healing	
2.3.5. Effect of stem cells on healing of oral -----	32
Ulceration	
3. Aim of the study-----	34
4. Materials and methods-----	35
1. Materials-----	35
Samples-----	35
Stem cells-----	35
Chemotherapy-----	35
Grouping of the animals-----	36
2. Methodology-----	36
Administration of chemotherapy-----	36
Induction of stomatitis-----	37
Injection of stem cells-----	38
Oral mucositis scoring system-----	39
Weighting the animals-----	40
Samples collection and processing-----	40
a) Haematoxylin and eosin stain-----	41
b) immunohistochemical marker-----	41
c) Fluorescent microscope-----	43
Digital image analysis-----	43
5. Results-----	40
Clinical results-----	40
1.Stomatitis examination-----	40

Group 1-----	50
Group 2-----	50
Group 3-----	51
Group 4-----	52
Statistics of OMS-----	53
2. Body weight loss-----	61
Statistical analysis of body weight loss--	66
Histological results -----	67
1. Hematoxyline & eosin stain-----	67
Group (1)-----	67
Group (2)-----	70
Group (3)-----	83
Group (4)-----	92
2. Immunohistochemical results-----	96
Group (1)-----	96
Group (2)-----	101
Group (3)-----	106
Group (4)-----	113
Statistical results of immunohistochemistry	119
3. Florescent microscope results-----	127
Group (1)-----	127
Group (2)-----	127
Group (3)-----	127
Group (4)-----	129
6. Discussion-----	131
7. Conclusion-----	144
8. Summery-----	140
9. References-----	149

## List of tables

<b>Table No.</b>	<b>Contents</b>	<b>Page</b>
<b>Table (1)</b>	Grading of oral mucositis	18
<b>Table (2)</b>	Summary of experiment steps	39
<b>Table (3)</b>	Oral mucositis scoring system.	40
<b>Table (4)</b>	Oral mucositis scoring system.	45
<b>Table (5)</b>	Number of samples for each OMS value in each group.	53
<b>Table (6)</b>	The mean values and standard deviation of OMS in each group	54
<b>Table (7)</b>	Paired comparisons between OMS mean values of each experimental group with control group at day 8 (sub groups A).	55
<b>Table (8)</b>	Paired comparisons between OMS mean values of each experimental group with control group at day 10 (sub groups B)	56
<b>Table (9)</b>	Paired comparisons between OMS mean values of experimental sub groups (2A, 3A and 4A).	57
<b>Table (10)</b>	Paired comparisons between OMS mean values of experimental sub groups (2B, 3B and 4B).	59
<b>Table (11)</b>	Paired comparisons between OMS mean values of day 8 and day 10 (subgroup A and B) in each group.	60
<b>Table (12)</b>	Weight monitoring of the animals	61
<b>Table (13)</b>	Mean values and standard deviation of the animals weight	64
<b>Table (14)</b>	Comparison between the animals weight at first and last day of the study in each group.	66
<b>Table (15)</b>	digital image analysis of PCNA expression in each group	119
<b>Table (16)</b>	mean and std. deviation of PCNA expression in each group	120
<b>Table (17)</b>	comparison of PCNA immunolocalization between the experimental groups and control group at day 8 (sub group A)	122

<b>Table (18)</b>	comparison of PCNA immunolocalization mean values between the experimental groups and control group at day 10 (sub group B)	123
<b>Table (19)</b>	Paired comparisons of PCNA immunolocalization mean values of sub groups (2A, 3A and 4A)	124
<b>Table (20)</b>	Paired comparisons of PCNA immunolocalization mean values of sub groups (2B, 3B and 4B).	125
<b>Table (21)</b>	Paired comparisons between PCNA immunolocalization mean values of day 8 and day 10 (subgroup A and B) in each group.	126

## **List of figures**

<b>Fig. No.</b>	<b>Title</b>	<b>Page</b>
<b>Fig. (1)</b>	Induction of stomatitis.	37
<b>Fig. (2)</b>	Intravenous injection of stem cells in the tail vein.	38
<b>Fig. (3)</b>	Florescent microscope used in the study	43
<b>Fig. (4)</b>	Digital image analysis using Video Test Morphology software	44
<b>Fig. (5)</b>	A photograph showing normal buccal mucosa (OMS = 0)	46
<b>Fig. (6)</b>	A photograph showing slight pink areas in buccal mucosa (arrows) (OMS= 0.5)	46
<b>Fig. (7)</b>	A photograph showing slight erythematous area in buccal mucosa (OMS= 1).	47
<b>Fig. (8)</b>	A photograph showing sever redness in buccal mucosa (arrow) (OMS= 2).	47
<b>Fig. (9)</b>	A photograph showing focal desquamation of buccal mucosa (OMS= 3).	48
<b>Fig. (10)</b>	A photograph showing exudation covering less than one half of buccal mucosa (OMS= 4).	48
<b>Fig. (11)</b>	A photograph showing virtually complete ulceration of buccal mucosa (arrow) (OMS= 5).	49
<b>Fig. (12)</b>	Column chart showing Difference between OMS mean valu each group.	49
<b>Fig. (13)</b>	Line chart monitoring the body weight in each group.	65
<b>Fig. (14)</b>	Column chart showing the animals weight at 1 <sup>st</sup> and 10 <sup>th</sup> day.	66
<b>Fig. (15)</b>	A photomicrograph of subgroup 1A showing continuous epithelial layer with intact basal cell layer (H&E x100).	69
<b>Fig. (16)</b>	A higher magnification of fig. 15 showing intact B.M. (H&E x200).	69
<b>Fig. (17)</b>	A photomicrograph of subgroup 1A showing: continuous basal cell layer, mitotic activity in the epithelium and inflammatory cells in the C.T. (H&E x200).	70
<b>Fig. (18):</b>	A photomicrograph of subgroup 1A showing: hyperkeratosis, mitotic activity in the epithelium and vasodilatation of blood vessels with interrupted endothelial lining and engorged with	70

	blood (H&E x200).	
<b>Fig. (19)</b>	A photomicrograph of subgroup 1A showing an ulcerated mucosa (H&E x100).	71
<b>Fig. (20)</b>	A higher magnification of fig. 19 showing degenerated epithelium, extravasated RBCs and inflammatory cells (H&E x200).	71
<b>Fig. (21)</b>	A photomicrograph of subgroup 1A showing granulation tissue: area of necrosis, inflammatory cells, discontinuity of endothelial lining of dilated bl. V. and extravasated RBCs (H&E x400).	72
<b>Fig. (22)</b>	A photomicrograph of subgroup 1B showing: intact basal cell layer (black arrows), C.T. slightly infiltrated with inflammatory cell (red arrows) (H&E x100).	72
<b>Fig. (23)</b>	Higher magnification of fig. 22 showing: intact B.M. , C.T. slightly infiltrated with inflammatory cell and dilated blood vessel filled with RBCs (H &E x200).	73
<b>Fig. (24)</b>	A photomicrograph of subgroup 1B showing: hyperkeratosis, intact B.M., connective tissue slightly infiltrated with inflammatory cell (H&E x100).	73
<b>Fig. (25)</b>	higher magnification of fig. 24 showing: hyperkeratosis, dilated blood vessel filled with RBCs, mitotic activity (H &E x200).	74
<b>Fig.(26)</b>	A photomicrograph of subgroup 2A showing ulcerated mucosa and discontinuity of basal cell layer (black arrows) (H&E x100).	77
<b>Fig. (27)</b>	Higher magnification of fig. 26 showing: granulation tissue (H &E x200).	77
<b>Fig.(28)</b>	A photomicrograph of subgroup 2A showing: ulcerated mucosa (H&E x100).	78
<b>Fig. (29)</b>	Higher magnification of fig. 28 showing ulcer edge; necrotic epithelium (blue arrows), absence of cells (rectangle) in C.T. and laceration of keratin in epithelium (red arrows) (H &E x200).	78
<b>Fig. (30)</b>	A photomicrograph of subgroup 2A showing: continuous epith. With lacerated keratin and C.T. infiltrated with inflammatory cell (H&E x100).	79
<b>Fig. (31)</b>	higher magnification of fig. 30 showing: (1) mitotic activity, C.T. infiltrated with inflammatory cells and congested bl. V. (H&E x200).	79

<b>Fig.(32)</b>	A photomicrograph of subgroup 2B showing: ulcerated mucosa and detached keratin. (H&E x40).	80
<b>Fig.(33)</b>	A photomicrograph of subgroup 2B showing: lacerated epithelium .(H&E x40).	80
<b>Fig.(34)</b>	higher magnification of fig. 33 showing lacerated epithelium, lacerated keratin and lack of nuclei from prickle and granular cell layer. (H&E x200).	81
<b>Fig.(35)</b>	Higher magnification of fig. 33 showing lamina propria: bl. V. engorged with blood, few Inflammatory cells. (H&E x200).	81
<b>Fig. (36)</b>	A photomicrograph of subgroup 2B showing: interrupted basal cell layer (red arrows), infl. Cells (blue arrows) and congested blood vessels (black arrow) (H&E x100).	82
<b>Fig.(37)</b>	higher magnification of fig. 36 showing: mitotic activity, and congested blood vessels(black arrow) (H&E x200)	82
<b>Fig.(38)</b>	A photomicrograph of subgroup 3A showing an ulcer site surrounded by degenerated epith. (H&E x100).	85
<b>Fig. (39)</b>	A higher magnification of fig. 44 showing : extravasated RBCs (black arrows) and (2) acute inflammatory cells (blue arrows) (H &E x400)	85
<b>Fig. (40)</b>	A photomicrograph of subgroup 3A showing: an ulcer surrounded by degenerated epithelium (H&E x100).	86
<b>Fig.(41)</b>	A higher magnification of fig. 40 showing: absence of nucluei in the epithelial cells, disorganization of basal cells, hyaline degeneration in C.T. and mild infiltration of inflammatory cells (H &E x200)	86
<b>Fig.(42)</b>	A photomicrograph of subgroup 3A showing: lacerated epithelium Covering C.T. with blood vessels engorged with coagulated blood (H&E x100).	87
<b>Fig. (43)</b>	A higher magnification of fig. 42 showing: lacerated epithelium, disorganization of basal cells, b.vs. engorged with coagulated blood and extravasated RBCs (H&E x200).	87
<b>Fig. (44)</b>	A photomicrograph of subgroup 3B showing an ulcer filled with granulation tissue (H&E x100)	88
<b>Fig.(45)</b>	A photomicrograph of subgroup 3B showing ulcer edge of fig. 51: disorganization of basal cell layer and inflammatory cells (H &E x200).	88

<b>Fig. (46)</b>	A photomicrograph of subgroup 3B showing an ulcer filled with necrotic tissue (H&E x40)	89
<b>Fig. (47)</b>	Higher magnification of fig. 46 showing ulcer edge: degenerated epithelium, chronic inflammatory cells and dilated bl. v. engorged with bl. (H &E x200).	89
<b>Fig. (48)</b>	Higher magnification of fig. 46 showing necrotic tissue of the ulcer: loss of cellularity , hyaline degeneration and inflammatory cells (H &E x200).	90
<b>Fig. (49)</b>	A photomicrograph of subgroup 3B showing complete necrosis and detachment of epithelium and heavy underlying granulation tissue (H&E x100)	90
<b>Fig.(50)</b>	higher magnification of fig. 49 showing: heavy inflammatory cells and congested bl. v. (H &E x200)	91
<b>Fig. (51)</b>	Photomicrograph of subgroup 3B showing ulcer healing site: fusion of basal cell layer , heavy infiltration of inflammatory cells (H&E x200).	91
<b>Fig.(52)</b>	A photomicrograph of subgroup 4A showing complete ulceration of mucosa (H&E x100).	93
<b>Fig. (53)</b>	A photomicrograph of subgroup 4A showing ulceration of mucosa , disorganization of basal cells and enlarged bl. v. engorged with coagulated blood (H&E x100).	93
<b>Fig. (54)</b>	photomicrograph of subgroup 4B showing: continuous epith., mitotic activity , numerous blood vessels (H &E x200).	94
<b>Fig. (55)</b>	Photomicrograph of subgroup 4B showing: disrupted basal cell layer, detachment of epith. and extravasation of RBCs (H &E x 200)	94
<b>Fig.(56)</b>	Higher magnification of fig. 55 showing : chronic inflammatory cells and and extravasated RBCs (H &E x400).	95
<b>Fig. (57)</b>	A photomicrograph of subgroup 4B showing: a small ulcer surrounded by healthy epithelium and dilated congested blood vessels. (H&E x100).	95
<b>Fig.(58)</b>	Photomicrograph of subgroup 1A showing the ulcer site (PCNA× 100).	98
<b>Fig.(59)</b>	Higher magnification of fig. 58 showing the ulcer edge: +ve reaction in basal and prickle cell layer , -ve reaction in granular cells and +ve reaction in C.T. (PCNA × 400).	98
<b>Fig.(60)</b>	Photograph of subgroup 1A showing: +ve nuclei in the basal and	99

	parabasal cells , few in prickle cells, -ve reaction in granular cell layer and +ve in C.T. (PCNA × 400).	
<b>Fig.(61)</b>	Photomicrograph of subgroup 1B showing the ulcer site (PCNA× 100).	99
<b>Fig. (62)</b>	Higher magnification of fig. 61 showing: -ve reaction in some areas and +ve reaction in other areas of basal cell layer, -ve reaction in prickle and granular cells, +ve reaction in C.T. (PCNA × 400).	100
<b>Fig. (63)</b>	Photograph of subgroup 1B showing: +ve reaction in basal and suprabasal cells , -ve reaction in prickle and granular cells, +ve reaction in C.T. (PCNA × 400).	100
<b>Fig. (64)</b>	Photomicrograph of subgroup 2A showing ulcer site ; -ve reaction in ulcer area and + ve reaction in ulcer edge (PCNA× 100).	103
<b>Fig. (65)</b>	Higher magnification of fig. 64 showing ulcer edge: +ve reaction in basal and prickle cell layer, -ve reaction in granular cells and +ve reaction in C.T. (PCNA × 400).	103
<b>Fig. (66)</b>	Photomicrograph of subgroup 2A showing: +ve nuclei in basal cells, -ve reaction in prickle, granular and C.T. cells (PCNA × 400).	104
<b>Fig. (67)</b>	Photomicrograph of subgroup 2B of lacerated epith. showing : few areas of +ve reaction in basal cell layer (PCNA× 100).	104
<b>Fig.(68)</b>	Photomicrograph of subgroup 2B showing: +ve reaction in the basal and suprabasal cells , -ve reaction in prickle and granular cells and +ve reaction in C.T. (PCNA × 400)	105
<b>Fig.(69)</b>	Photomicrograph of subgroup 2B showing: +ve reaction in basal and suprabasal cell layer and -ve reaction in basal , prickle and granular cells and and +ve reaction in C.T. (PCNA × 400).	105
<b>Fig.(70)</b>	Photomicrograph of subgroup 3A showing ulcer site: -ve reaction in ulcer area, +ve reaction in surrounding epithelium and C.T. (PCNA× 100).	108
<b>Fig.(71)</b>	Higher magnification of fig. 70 showing ulcer edge: +ve reaction in basal and prickle and C.T. cells, -ve reaction in prickle cells (PCNA × 400).	108
<b>Fig.(72)</b>	Photomicrograph of subgroup 3A showing: few +ve reaction in basal cells, -ve reaction in basal, prickle and C.T. cells (PCNA × 400).	109
<b>Fig. (73)</b>	Photomicrograph of subgroup 3B showing ulcer site: +ve reaction in the ulcer granulation tissue (PCNA× 100).	110

<b>Fig.(74):</b>	Photomicrograph of subgroup 3B showing ulcer edge: +ve reaction in the ulcer area and the epithelium (PCNA× 100).	110
<b>Fig.(75)</b>	Higher magnification of fig 74 showing: +ve reaction in the basal and prickle cells, -ve reaction in prickle and granular cells. +ve reaction in the C.T. (PCNA × 400).	111
<b>Fig.(76):</b>	Photomicrograph of subgroup 3B showing ulcer site: +ve reaction in basal cells and and C.T. cells (PCNA× 100).	111
<b>Fig. (77)</b>	Higher magnification of fig 76 showing: +ve reaction in the basal cells and -ve reaction in prickle and granular cells.(PCNA × 400).	112
<b>Fig.(78)</b>	Photomicrograph of subgroup 4A showing ulcer site: -ve reaction in the ulcer area and +ve reaction in the ulcer edge (PCNA× 100).	115
<b>Fig.(79)</b>	Higher magnification of fig 78 showing ulcer edge: +ve reaction in the basal and prickle cells, -ve reaction in coagulation areas of C.T. (PCNA × 400).	115
<b>Fig.(80)</b>	Photomicrograph of subgroup 4A showing: +ve reaction in basal cells, –ve reaction in prickle cells and some areas of basal cells, +ve reaction in C.T. (PCNA × 400).	116
<b>Fig.(81):</b>	Photomicrograph of subgroup 4B showing ulcer site : -ve reaction in the ulcer area and +ve reaction in the ulcer edge (PCNA× 100).	117
<b>Fig.(82)</b>	Higher magnification of fig 81 showing ulcer edge: +ve reaction in the basal cells , -ve reaction in prickle and superficial cells (PCNA × 400).	117
<b>Fig.(83)</b>	Photomicrograph of subgroup 4B showing: +ve reaction in the basal and suprabasal and C.T. cells, -ve reaction in prickle cells (PCNA × 400).	118
<b>Fig. (84)</b>	Column chart showing means of PCNA expression in each group	121
<b>Fig.(85)</b>	Photomicrograph of subgroup 3A showing: aggregates of MSCs labeled with PKH 26	128
<b>Fig.(86)</b>	Photomicrograph of subgroup 3B showing: few aggregates of MSCs labeled with PKH 26 .	128
<b>Fig.(87)</b>	Photomicrograph of subgroup 4A showing: many aggregates of MSCs labeled with PKH 26	130
<b>Fig.(88)</b>	Photomicrograph of subgroup 4B showing: few aggregates of MSCs labeled with PKH 26.	130

## List of abbreviations

<b>Abbreviation</b>	<b>Meaning</b>
<b>5-FU</b>	5-Fluorouracil
<b>FUMP</b>	5-fluorouridine monophosphate
<b>GIT</b>	gastrointestinal tract
<b>OM</b>	oral mucositis
<b>Gy</b>	Gray: is the unit of absorbed dose, which represents the amount of radiation required to deposit one joule of energy in one kilogram of any kind of matter
<b>NCI-CTC</b>	National Cancer Institute–Common Toxicity Criteria
<b>WHO</b>	World Health Organization
<b>IA</b>	intra-artery
<b>IV</b>	Intravenous
<b>IP</b>	intraperitoneal
<b>MSCs</b>	mesenchymal stem cells
<b>BM-MSCs</b>	bone marrow mesenchymal stem cells
<b>FGF</b>	fibroblast growth factor
<b>VEGF</b>	vascular endothelial growth factor
<b>BMSCs</b>	bone marrow stem cells
<b>hMSCs</b>	Human mesenchymal stem cells
<b>OMS</b>	oral mucositis scoring system
<b>H&amp;E</b>	<u>Haematoxylin and Eosin</u>
<b>PCNA</b>	Proliferating Cell Nuclear Antigen
<b>IR</b>	Infrared
<b>GMSCs</b>	gingival mesenchymal stem cells
<b>hGMSC</b>	human gingival mesenchymal stem cells
<b>hASCs</b>	human adipose stem cells
<b>GvHD</b>	graft-versus-host disease

# **1. Introduction**

With their tremendous clinical utility, there are many classes of chemotherapeutic agents in the market that are used effectively as anti-neoplastic treatment (**Jaime et al., 2003**).

The effective treatment of malignancies is usually limited by its harmful effect on normal, healthy cells. Mucosal cells in the gastrointestinal tract (from the mouth to anus) are highly susceptible to the toxic effects of cancer treatment. These toxicities (including mucositis and stomatitis) are some of the most significant unavoidable effect associated with cancer treatment. So, Stomatitis (the mucositis of oral mucosa) is the best-characterized manifestation because it results in symptoms in an area accessible to routine examination (**Denham et al., 1991**)

The goals of mucositis management are to prevent or reduce the severity of the drug toxicity and to manage the associated symptoms. Mucositis management allows the continued delivery of treatment without interruption or dose reduction leading to improvement of overall prognosis. But unfortunately there is no standard protocol that is used to completely cure or prevent oral mucositis (**Clarkson et al., 2010; Lalla et al., 2014**).

Stem cells are a subject of interest as a potentially revolutionary new way to treat diseases and injuries, with wide-ranging medical benefits as wound healing, ulcer treatment and management of side effects of chemotherapy (**Aboushady et al., 2012**).

## **2. Review of literature**

### **2.1. Chemotherapy:**

#### **2.1.1 Mechanism of action:**

Chemotherapeutic drugs cause damage to cells so, they are termed cytotoxic drugs. Most chemotherapeutic drugs work by impairing mitosis (cell division) (specially targeting fast-dividing cells). They prevent mitosis by various mechanisms including damaging DNA and inhibition of the cellular organelles involved in cell division. One theory explaining why these drugs kill cancer cells is that they induce a programmed cell death known as apoptosis (**Makin and Hickman, 2000; Malhotra and Perry, 2003**)

The process of cell division, whether normal or cancerous, passes through the cell cycle. The cell cycle goes from the resting phase, active growing phases, and then to mitosis. Chemotherapeutic drugs that affect cells only when they are dividing are called cell-cycle specific. While those affect cells when they are at rest are called cell-cycle non-specific. The scheduling of chemotherapy is set based on the type of cells, rate of cell division, and the time at which the given drug is likely to be effective. This is why chemotherapy (cell cycle specific ) is typically given in cycles. The main action of these drugs works against cells in a specific phase of the cell cycle; For example, the drug Cyclophosphamide acts upon cells in the DNA replication phase of the cycle, while other drugs, such as Taxol, more effectively influence cells in the division phase. These types of drugs do not affect cells in the