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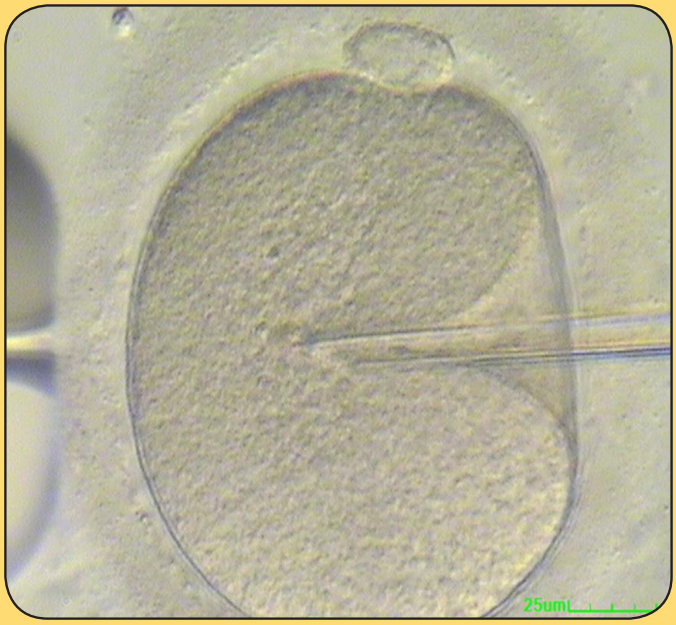

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We welcome journal submissions throughout the year but preferably by **March** and **September**. Articles submitted for publication are understood to be offered only to *JUMMEC* and which have not been sent to other journals for consideration.

Cover

Intracytoplasmic sperm injection (ICSI) in which a single human sperm is directly microinjected into a human oocyte. The individual sperm can be seen towards the sharp end of the glass micropipette about to be transferred into the oocyte cytoplasm. Image courtesy of Tracey Griffiths (Oxford Fertility Unit)

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The **Journal of Health and Translational Medicine (JUMMEC)** publishes both basic and applied science as well as clinical research studies on any area of medicine that is of interest and relevance to the medical community. This is a peer-reviewed journal that publishes Reviews Articles, Original Articles, Short Communications, Clinico-pathological Conference Abstracts, Case Reports, Letters to the Editor and Book Reviews.

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Foreword from the Editor-in-chief

Dear Readers of JUMMEC,

Welcome to the Journal of Health and Translational Research's *(JUMMEC)* 2nd issue published in the year 2013. In this issue, we are happy to present to you several interesting articles for your reading pleasure. More importantly, we hope the contents would be useful as a reference for your future research work. In this issue, we have included reports by two research teams working on tissue engineering and stem cell research in Malaysia. The areas of tissue engineering and stem cells are emerging in Malaysia, although at present, these areas of research are not as well established as many others, such as those in the areas of infectious diseases or molecular medicine. However, this is a start and we hope that many more of such articles would be published in our journal or other local journals in the near future. Also covered in this issue is the report by Suseela Yelumalai, who is currently studying in University of Oxford, describing her research work and the potential collaboration will be undertaken with University of Malaya in the next few years.

However, the main feature of this issue is an article written by Prof. Dato' Dr. Tunku Sara Tunku Ahmad, which describes her ongoing effort in doing research and providing clinical services in University of Malaya as a Hand and Micro-surgeon for more than 20 years. In her article, Prof. Sara has highlighted the improvements made in the provision of medical services and how the customary practice of surgery have been transformed to more effective means of providing healthcare in an evidence-based manner. The article based on her inaugural lecture in 2008 and, is clearly an inspiration to many of us. I have had the privilege of being mentored by this brilliant teacher, and it is with great pleasure that I feature the article by Prof. Sara as the spotlight article in this issue.

I hope that the articles featured in this issue will inspire the readers to pursue better research in an attempt to strive for excellence in areas of health and translational medicine.

With best wishes,

Tunku Kamarul Zaman,
Editor-in-chief,
The Journal of Health and Translational Medicine.

ADIPOSE-DERIVED STEM CELLS IN TISSUE ENGINEERING: LABORATORY TO BEDSIDE

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ABSTRACT

Human adipose tissue has been recognized as an alternative source of adult stem cells. The abundance and ease of harvest of adipose tissue has made it suitable for use in regenerative medicine and tissue engineering. Adipose-derived stem cells isolated from human adipose tissue are able to differentiate into several mesenchymal lineages and secrete growth factors that exhibit therapeutic potential. Protein profiles have been established using various isolation methods, which has expanded researchers' understanding of adipose-derived stem cells in clinical applications. This review highlights the properties, isolation methods, immunophenotype and clinical applications of adipose-derived stem cells.

Keywords: *adipose-derived stem cells, immunophenotyping, isolation methods, clinical applications*

Introduction

Advances in the fields of biomedical engineering, biochemistry, genetics, material science, cell biology and molecular biology have given rise to the remarkable new cross-disciplinary field of tissue engineering. Large-scale human and animal cell cultures that include skin, cartilage, muscle, bone, endothelial and stem cells have been studied and clinically applied to replace damaged tissues or organs in humans. This new branch of modern medicine has been termed regenerative medicine. Regenerative medicine combines several factors, including cells, growth factors and biomaterials. Strategies include cell therapy, biomaterials alone and combinations of cells and biomaterials (1). When implanted in the body as temporary structures, naturally derived or synthetic biomaterials can provide templates that guide tissue formation while the scaffold is gradually biodegraded. Stem cells for regenerative medicine should have the following criteria: they (a) are abundantly present, (b) can be harvested using minimally invasive procedures, (c) are able to reproducibly differentiate into multiple cell lineages, (d) can be safely and effectively transplanted to either an autologous or allogeneic host, and (e) can be manufactured in accordance with current Good Manufacturing Practice (GMP) guidelines (2).

Generally, stem cells are unspecialized cells that are able to multiply themselves or become specialized types of cells. Stem cells can be divided into several classes according to potency; namely, totipotent stem cells can build an entire organism, and pluripotent stem cells can become all tissue types except for extraembryonic tissues such as placenta and amnion. Multipotent stem cells are able to develop into more than one cell type in the body. Cells that can differentiate into only one cell type are known as precursor cells or progenitor cells (3). Bone marrow is a recognized source of mesenchymal stem cells for regenerative medicine. Mesenchymal stem cells (MSC) from bone marrow are aspirated from patients using painful procedures and with relatively lower yields of cells than MSCs from adipose tissue (4).

Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst during embryogenesis. ESCs were first isolated from mouse embryos in 1980 and then later from humans in 1998 (5). Despite their pluripotency, humanity and ethics concerns relating to the destruction of embryos to obtain ESCs have been raised by various religious and research institutions worldwide. Differentiated cells reprogrammed to stem cells, called induced pluripotent stem (iPS) cells, are another interesting source of stem cells. Typically, iPS cells are produced by transfecting differentiated or adult cells with stem cell-associated genes. Thus, their properties are remarkably similar to ESCs (6).

Adult stem cells can be isolated from a mature organ such as bone marrow, adipose tissue, dermis, trabecular bone, periosteum, pericytes, blood and synovial membrane (7). These stem cells usually replicate to replace the dead cells of their origin organ. Thus, their multi-potency to differentiate into other lineages can be employed in stem cell-based therapy. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy provided several guidelines to define human mesenchymal stromal cells, such as adherence to plastic culture flasks; expression of specific antigens/ markers such as CD105, CD73 and CD90; and multipotent *in vitro* multi-lineage differentiation into osteoblasts, adipocytes and chondroblasts (8).

Adipose tissue and adipose-derived stem cells

Adipose tissue modulates energy homeostasis and secretes lipids and proteins, as well as peptides such as hormones, adipokines and cytokines (9). The majority of adipose tissue in mammals can be found in the subcutaneous and intra-abdominal areas (10). Subcutaneous fat is the fat present below the skin, and intra-abdominal fat is omental, mesenteric and perirenal. There are two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). White adipose tissue is predominantly present in adults, while brown adipose tissue is present in newborns. The largest deposits of WAT are in the subcutaneous and visceral areas (11). Based on structural and ultra-structural features, three types of subcutaneous WAT have emerged: deposit WAT (dWAT), structural WAT (sWAT) and fibrous WAT (fWAT) (12).

Adipose tissue originates from the mesodermal layer during embryogenesis. Adipose tissue is vascularized by a capillary network of blood vessels during development. Mature adipocytes are filled with a single lipid droplet interlarded with stroma, which is composed of fibroblasts, blood vessels, leukocytes, macrophages and preadipocytes (lacking the lipid droplet) (13). Approximately 10% of fat cells are renewed each year, irrespective of body mass index and at all adult ages (14). As adipocytes are a terminally differentiated cell, their replacement with new adipocytes to compensate for lost adipocytes originates from a population of progenitor cells (pre-adipocytes) that are present in the stromal fraction of the adipose tissue (15, 16).

Adipose tissue progenitor cells were initially thought to only differentiate into adipocytes. However, these cells have gained greater attention through recent research work showing that cells isolated from lipoaspirate have multipotential ability *in vitro* to differentiate outside of the adipogenic lineage, including differentiation to chondrogenic, myogenic, and osteogenic cells via the use of specific growth medium induction (17). Therefore, adipose tissue harvested with minimal pain and great availability from surgical operations presents an alternative and promising source of stem cells for use in regenerative medicine. Many terms have been used in the literature to refer to cells isolated from adipose tissue, including adipose derived stem/stromal cells (ASCs), adipose–derived adult stem (ADAS) cells, adipose-derived adult stromal cells, adipose-derived stromal cells (ADSC), adipose stromal cells (ASC), adipose mesenchymal stem cells (AdMSCs), lipoblasts, pericytes, pre-adipocytes, and processed lipoaspirate (PLA) cells (18). To avoid confusion, the International Federation for Adipose Therapeutics and Science (IFATS) agreed to adopt the term ‘‘adipose-derived stem cells” (ASCs) to identify the isolated, plastic-adherent and multipotent cell population (18).

ASCs from adipose tissue have similar multipotency to that of MSCs from bone marrow (4). The population of ASCs may partly originated from pericytes and fibroblasts from surrounding adipose tissue environment (19). Pericytes are cells that line endothelial cells in capillaries and microvessels. Pericytic markers are expressed in ASCs cultures, implying that ASCs cultures may originate from pericytes resident in proximity to adipose tissue (20, 21). However, native ASCs do not express *in situ* pericytic markers, and ASCs can be found scattered in adipose tissue stromal (16).

The stromal vascular fraction (SVF) is the pellet obtained from collagenase digestion of adipose tissue. It can be composed of a heterogeneous cell population that includes pre-adipocytes, endothelial cells, fibroblasts, smooth muscle cells, pericytes, leukocytes and mast cells (22). However, cultured SVF displayed a fibroblast-like morphology and was maintained *in vitro* long term with a low level of senescence (17, 23).

There is increasing evidence that ASCs can be differentiated into mesodermal lineages, including adipogenic (24, 25), osteogenic (24, 26), chondrogenic (27, 28) and myogenic (29, 30) lineages in their respective growth medium. Moreover, ASCs can also be differentiated into non-mesodermal lineages, such as endothelial cells (24, 31), neuronal cells (32), epithelial cells (33, 34), pancreatic cells (35) and hepatocytes (36). Despite their multipotency, ASCs secrete several angiogenic and antiapoptotic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which may act synergistically for cardiovascular protection (37).

In vitro study has shown that ASCs promote dermal wound healing by increasing collagen synthesis, thus promoting fibroblast proliferation via the paracrine activity of ASCs secretory factors (38). *In vivo* study has shown that ASCs significantly reduce wound size in mice (38). Furthermore, factors secreted by ASCs produce a whitening effect by inhibiting melanin synthesis (39). ASC-conditioned medium has antioxidant properties because they protect human dermal fibroblasts from oxidative injury (40). Hair growth is stimulated upon treatment with the growth factors secreted by ASCs (41). In addition to wound healing, ASCs also have anti-ageing properties that act by stimulating collagen synthesis and angiogenesis (42).

In reconstructive surgery, skin flaps are used to repair defects caused by injuries and other causes. A lack

of blood supply results in necrosis of the flap tissue. New blood vessel formation and the elongation of existing blood vessels are two mechanisms that assist revascularization. Newly formed blood vessels originating from the differentiation of ASCs into endothelial cells and the secretion of angiogenic factors improve the viability of the skin flap (43, 44).

In vitro isolation and propagation technology for ASCs

Primary cultures are created when cells grow out from an explants or attach to substrate after enzymatic or mechanical disaggregation (45). Primary cultures can be defined as a mixture of cells or highly purified cells isolated directly from organism. The function of cells from a primary culture is expected to resemble that of *in vivo* cells (46). The cell isolation procedure is performed in a class II biosafety cabinet to minimize contamination. There is no standard protocol for the isolation of human ASCs. Generally, adipose tissue is enzymatically digested, centrifuged and filtered (see Figure 1). Factors including heterogeneity between patients, tissue depot, concentration and collagenase type, centrifugation and media formulations may result in variability in the yield and *in vitro* characteristics of the cells. These factors may affect marker expression, differentiation capability and therapeutic potential.

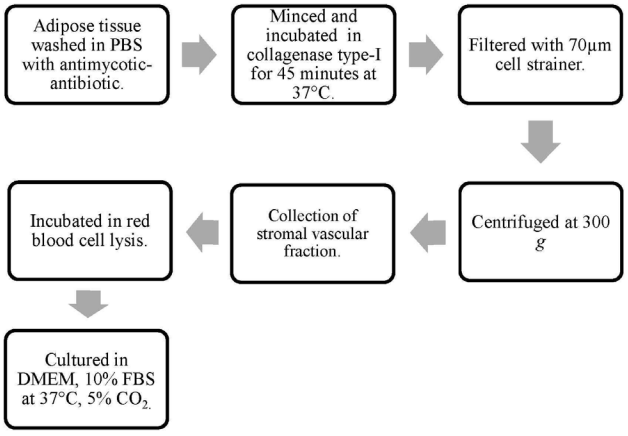

Figure 1: Isolation of ADSCs from human adipose tissue. Abbreviations: PBS: phosphate buffered saline, DMEM: Dulbecco's modified eagle medium, FBS: fetal bovine serum.

One of the ways to isolate cells from tissue is by enzymatic disaggregation. Tissue is washed with phosphate-buffered saline to remove debris and blood. It is then minced and incubated in collagenase with continuous rotation. Collagenase is widely used as an enzyme to help disperse the collagenase bundles that are present in the extracellular matrix and thus dissociate cells from tissue. Many types of digestion buffer have been used to digest the tissue, including collagenase type I (18) and collagenase with trypsin (23). The optimal collagenase concentration and digestion time are 0.2% and 1 hour, respectively (47). The combination of collagenase with trypsin can shorten the time of incubation from 3 hours to 20 minutes (23). Trypsin is used to dissociate the cells. After incubation, the activity of collagenase and trypsin can be neutralized with growth medium containing serum. Collagenase is less aggressive than trypsin (48). The presence of erythrocytes can reduce ASCs adherence and proliferation (49). Therefore, erythrocyte cross-contamination can be overcome by lysing the red blood cells in lysis buffer. However, erythrocytes are also non-adherent to plastic, allowing primarily ASCs to attach.

The cells are centrifuged at 180 *g* for 5 minutes and filtered through a 70-µm cell strainer to obtain a single cell suspension and remove the undigested tissue. Centrifugation is not a precise cell separation technique; however, it concentrates the cells and removes fractions of cells based on density. Two measurements of centrifugal force are used: rpm (revolutions per minute) and *g* (relative centrifugal force). The same centrifugal force may correspond to different rpm values among researchers if they have different radii of centrifugation. Therefore, specific gravity is used for standardization. Centrifugation of adipose tissue at more than 3000 *g* significantly damaged the adipose-derived stem cells, and the number of ASCs remained unchanged at 1200 *g* (50). Another finding suggested 1126 *g* as the optimal centrifugal force in comparison to 12,519 *g* and 4507 *g*, which resulted in more viable ASCs (51). To date, there is no extensive research on the relationship of various centrifugal forces on the yield of ASCs.

ASCs in the culture appear spindle-shaped or fibroblast-like (17, 23, 52). When 70-80% confluence is reached, the cultured cells need to be further propagated in additional vessels, a procedure called sub-culture. Cells can be detached from the culture flask by incubating the cells in dissociating agents, such as trypsin, for 3-5 minutes at 37°C. Culture flasks can be tapped to assist with cell detachment. Detached cells usually float and exhibit a round shape in the culture medium.

A simple and fast method to quantify viable cells utilizes the trypan blue exclusion assay. Viable cells with intact membranes do not take up the blue dye; thus, dead cells with blue color can be distinguished from viable cells. The expansion of cells *in vitro* requires growth medium. Growth medium provides necessary nutrients, growth factors and hormones, and it regulates the culture pH and osmotic pressure. This medium is a combination of basal media and 10-20% of serum. Basal media is a media that contains nutrients such as amino acids, vitamins, inorganic salts and a carbon source, such as glucose. The most frequently used basal media for ASC culture are Dulbecco's modified Eagle medium (DMEM) (35, 53-57), DMEM/F12 (58-62) and alpha-modified Eagle medium (α-MEM) (63). The expansion of ASCs in α-MEM is significantly better than the expansion in DMEM, DMEM/F12, F12 and LP02 (commercial media free from animal product) when all are supplemented with 10% serum (64). This parameter

is evaluated based on the differentiation capacity of ASCs rather than the immunophenotype of the ASCs (64). Laboratory-made media composed of basal media, several growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), and serum have preserved the stemness of ASCs, as measured by their proliferation and expression of stemness transcriptional markers (49). However, human MSC stemness from bone marrow is better preserved in DMEM than in Iscove's modified Dulbecco's medium (IMDM) (65). High calcium medium can cause cell differentiation. Low calcium medium can accelerate proliferation and prolong their lifespan (66).

ASC stemness should be preserved in both *in vitro* and *in vivo* situations for optimal effects in regenerative medicine. The most common serum used is animal-derived, such as fetal bovine serum and fetal calf serum. Sera from animals may cause contamination and are expensive. Therefore, researchers are looking into new alternative formulations of media, such as serum-free media or animal-free sera (67-69). In addition, we can avoid transmission of diseases derived from animals if ASCs are employed clinically in humans. Similar results are obtained when porcine-derived trypsin is compared with animal protein-free products based on yield, viability and immunophenotype of the human ASCs (70). There is also a similar result for *in vitro* and *in vivo* proliferation and differentiation of human skeletal-derived MSCs when cultured in human serum and fetal bovine serum (71). Another study states that ASCs cultured in human allogeneic serum and fetal bovine serum have a similar surface marker phenotype but differ in gene expression after differentiation (72). Artificial serum substitute induces more profound cellular physiological changes in comparison to ASCs grown in fetal calf serum, pretested fetal calf serum or human allogeneic serum (73). Despite the fact that animal-free serum such as human serum may be a great alternative, mass production of serum from healthy humans may be needed for long term ASC culture.

The site of tissue harvesting may influence the concentration and function of human ASCs. Lipoaspirates from the inner thigh and lower abdomen may have a higher concentration of MSCs than lipoaspirates harvested from the upper abdomen, trochanteric region, knee, and flank (74). Different locations on the same individual may yield different amounts of stem cells. Subcutaneous adipose tissue from the hip has more stem cells than the subcutaneous space of the abdomen (75). This finding contradicts the results of a study, which found that subcutaneous adipose tissue abdomen yields more ASCs than adipose tissue from the hip or thigh region (22). In terms of gender, the abdomen has the highest cell yield in males compared to the knees and back region, whereas there is no significant relationship between the collection site and cell yield in females (47). The osteogenic differentiation capability of ASCs from superficial and deep fat and the dependence on gender and age have been examined. ASCs from the superficial layer of fat from males differentiate into osteocytes faster than those from the deep layer, while there is no significant differences of osteogenic properties of both depot in female (76). In humans, there are no data supporting which type of adipose tissue has greater plasticity. Nevertheless, subcutaneous structural WAT sites that have weak collagenic bundles and are rich in stem cells are preferable to sources of deposit WAT and fibrous WAT (12). However, in mice, inguinal WAT has shown greater differentiation potential than BAT (77).

There is inconsistency among researchers regarding the influence of age and body mass index (BMI) on the proliferation of ASCs. ASCs from younger donors have a higher proliferation rate than those from older donors (78). However, a study has found no significant relationship between age and the proliferation rate of ASCs (79). There is no significant correlation between the frequency of adipose-derived stem cells and BMI (22), and there are no statistical correlations between age and BMI and the proliferation and yield of ASCs (47, 80). Different isolation methods, culture conditions and sites from which adipose tissue is taken may cause variation between studies.

In addition to the manual isolation of cells, there is an automated device called the Celution™ system that is designed to wash, digest and concentrate cells from adipose tissue. These isolated cells comprise a heterogeneous population that contains ASCs, endothelial cells and vascular smooth muscle cells. The cells isolated by a Celution™ system possess equivalent expression results for certain markers and equivalent differentiation ability to cells isolated by the manual method (81). In comparison to enzymatic isolation, the mechanical isolation of cells from lipoaspirates can reduce the cost of collagenase and time of digestion while maintaining their antigen expression and differentiation capacity (82). ASCs can also be derived from mature adipocytes by dedifferentiating them into lipid-free fibroblast-like cells (83, 84).

There are heterogeneous populations of cells that may adhere to the plastic surfaces of a culture flask after the enzymatic aggregation of tissue**.** The isolation of ASCs by immuno-magnetic beads prior to culturing can reduce cross-contamination with unwanted cells. The cells are incubated with beads that are conjugated with MSC-associated antibodies, such as CD 105, CD 90 and CD73. The magnetically labeled cells are retained within the magnetic field in the column and collected by flushing out the cells after removing the column from the magnetic field. The isolated cells can be cultured, and the attached beads are biodegradable. The immuno-magnetic bead separation of cells after collagenase digestion of adipose tissue results in several subpopulations that share typical marker expression (85). Three different isolation methods have been studied to reduce the heterogeneity of cultures; these methods are based on adherence on plastic, washing after one hour of cell seeding and immuno-magnetic isolation by specific markers. Washing after one hour of seeding significantly reduces the heterogeneity of the cell population and increases stem cell marker expression. A more homogenous population is achieved when loosely attached cells are washed away after one hour of seeding instead of changing the medium after 24 hours or more (86).

Many protocols have been developed for the cryopreservation of ASCs. Cryopreservation requires a medium and a cryoprotectant. Fetal bovine serum prevents the viability loss of ASCs, and cryoprotective agents such as dimethylsulphoxide prevent cell death due to extreme temperature. ASCs can be cryopreserved for at least 6 months while maintaining their proliferation capability and surface marker profiles (87). Storage of liposuction samples for 24 hours at 4°C before processing the samples could increase the yield of stem cells (88).

Immunophenotyping of ASCs

In addition to adhering to plastic, stem cells must express specific surface antigens or proteins. The expression of specific antigen is crucial for clinical applications. Immunophenotyping of ASCs can be performed at various stages of culture (freshly isolated cells, early passages and late passages) and using various experimental methods (western blot, flow cytometry, immunofluorescence and immunocytochemistry). These methods offer either quantitative or qualitative expression of antigen. The depot of adipose tissue and type of sample (liposuction or excised adipose tissue) may influence the percentage of expression.

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed that more than 95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (less than 2% positive) of CD45, CD34, CD 14 or CD11b, CD79a or CD19 and HLA class II (8). Positive and negative markers of ASCs are summarized according to the publication in which they appear (see Table 1).

Table 1: Positive and negative markers of ASCs, as reviewed:

Positive	Negative/ Very Low
CD29 (23, 89, 90, 109, 110)	CD31 (22, 89, 90, 109, 111)
CD44 (23, 90, 109-111)	CD45 (22, 23, 89, 90, 109, 111, 112)
CD49d (89, 111)	CD34 (23, 90, 91, 109, 111)
CD73 (89, 90, 109, 110, 112)	CD106 (22, 109, 111)
CD90(22, 89, 109-112)	HLA-II (109)
CD105 (22, 23, 89, 90, 109-113)	CD14 (109)
CD13 (23, 111, 113)	CD44 (110)
CD166 (22, 23)	HLA-DR (22, 23, 90, 91)
CD34 (22)	CD146 (22)
HLA-ABC (22)	CD117 (91)
CD71 (111)	

ASCs express surface markers such as integrins (CD29, CD49d), surface enzymes (CD73), extracellular matrix proteins (CD90 and CD105) and hyaluronate (CD44). In contrast, ASCs do not express hematopoietic progenitor cell marker (CD34), vascular cell adhesion molecule (CD106), monocyte differentiation antigen (CD14), endothelial cell adhesion molecule (CD31) and leukocyte common antigen (CD45). Katz et al. demonstrated that ASCs strongly express proteins related to cell adhesion, matrix proteins, growth factors and receptors, and proteases (58).

Most papers report that cultured ASCs exhibit significantly increased expression of positive markers in the subsequent cultures (subcultures) (89-91). High expression of CD105, which is an MSC marker, has indicated that these cells can be applied to stem cells therapy (89). In contrast, decreased expression of CD105 is related to the differentiation of MSC into osteogenic, adipogenic, chondrogenic and epithelial pathways (92). CD34, CD31, CD45 are highly expressed in freshly isolated cells, and most reports claim that their expression decreases with successive cultures. This finding implies that the population of ASCs becomes more homogenous with increasing sub-cultures. Positive expression may be correlated with heterogeneous cell population in freshly isolated cells (SVF), which are composed of ASCs, endothelial cells and vascular smooth muscle cells (81). While CD34+ ASCs have greater proliferation potential, CD34- ASCs exhibit greater ability to differentiate into mesenchymal lineages (93).

ASCs share the same profile of antigen expression as MSCs from bone marrow: CD29, CD13, CD44, CD 73, CD90, CD105 and CD166 (59, 94). The differences between ASCs and bone-marrow-derived MSC are the expression of CD106 (only expressed by bone-marrow-derived MSCs) and CD 49d (only expressed by ASCs) (95). Mature fibroblasts in connective tissue also express CD29, CD44, CD90, CD105 and are negative for CD34, CD117 and CD146 (96).

Current clinical applications of ASCs

The multipotential nature and secretion ability of ASCs encourage to their clinical application. Here, we discuss the application of ASCs to soft tissue reconstruction and augmentation, wound management (radiation injury) and orthopedic clinical applications.

Soft tissue reconstruction and augmentation

Adipose tissue has been utilized as an autologous tissue for soft tissue augmentation to repair congenital and acquired tissue damage. To survive, the graft must be vascularized and supplied with adequate nutrients and cell sources. Differentiated adipocytes from autologous ASCs were injected into the depressed scars of thirty-one patients, and long-term follow up in seven patients revealed that the volume of the graft was maintained for up 12 weeks compared to conventional fat transfer (97). The injection of ASCs alone was not sufficient to support adipogenesis; thus, adipocytes differentiated from ASCs enhanced adipose tissue formation (98). Another novel method to enhance survival of graft was achieved by combining autologous fat with fat containing a stromal vascular fraction. This technique is known as cell-assisted lipotransfer (CAL),

which improves the survival rate of the graft and enhances angiogenesis in breast tissue augmentation and facial lipoatrophy (99, 100). ASC differentiation to adipocytes and vascular endothelial cells, secretion of growth factors and replication are important events that contribute to adipose tissue regeneration, angiogenesis and graft survival in CAL treatment (101). ASCs can be employed allogeneically because they possess immunosuppressive properties (lack of HLA-DR expression) (102, 103).

Wound management (Radiation injury)

Autologous lipoaspirate administered into lesions caused by radiation therapy treatment of oncologic patients improved the regeneration of tissue and the restoration of function. ASCs have been reported to actively participate in the regeneration and neovascularization of tissue (104).

In Japan, autologous non-cultured ASCs isolated by the Celution™ System were used as a treatment for local chronic injuries caused by radiation. The treated injuries were caused by nuclear power plant accidents or radiation therapy. In the conventional method of treatment, multiple surgical procedures must be performed before the wound can heal. Autologous non-cultured ASCs applied to the wound area with artificial dermis and sprayed with growth factors resulted in improved tissue regeneration after 1.5 years (105).

Orthopaedic

Calvarial fracture in children was treated with autologous ASCs in autologous fibrin glue. After three months, the fractures showed ossification of the defect (106). Another study also employed autologous ASCs isolated according to GMP guidelines and expanded *in vitro* using autologous serum. These cells were combined with beta-tricalcium phosphate and scaffold construct seeded into the rectus abdominal muscle of patients with hard palate defects. Bone formation and vascularization of tissue occurred after 8 months (107). ASCs were induced to osteocytes and implanted at surgically produced defects in the palatal bones of rats to examine the ability of differentiated ASCs to treat bone defects. The group treated with ASCs and scaffold exhibited significant bone regeneration (108). ASCs offer alternative treatments to traditional bone grafting, and this method is suitable when there is a shortage of autogenous bone, particularly in the cases of children and donor-site morbidity.

Conclusions and future directions

ASCs possess significant potential in regenerative medicine. ASCs have captured researchers' attention because, within 10 years, the investigation of their applications has progressed from bench to bedside. Numerous methods of isolation and culture conditions have been manipulated to obtain optimum ASCs expansion. Further study and standardization of the isolation protocol and antigen profiles would substantially enhance clinical advancement. ASCs have antioxidant, anti-aging, anti-inflammatory and wound healing properties, as demonstrated *in vitro* and in preclinical models. Cell therapy using ASCs can reduce morbidity and the healthcare cost burden to sustain their development in countries without compromising safety issues.

Basic science knowledge of ASCs can be expanded by further investigation of ASC biology. These findings may facilitate researchers' understanding of the *in vitro* behavior of these cells, which can translate into clinical applications. ASCs production potentially benefitting regenerative medicine should be performed in accordance with the provided code of practice. Further clinical trials are necessary to continue validation of the applications of ASCs and improve their clinical outcome.

*For literature searching, authors key in "adipose-derived stem cells", "mesenchymal stem cells", "adipose tissue", "characterization of adipose-derived stem cells", "isolation of adipose-derived stem cells", "clinical application of adipose-derived stem cells" in PubMed portal and Science Direct.

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AN INTRODUCTION TO PROF. DATO' DR. TUNKU SARA TUNKU AHMAD

Tunku Sara binti Tunku Ahmad Yahaya, Professor of Orthopaedic Surgery and member of the unit of upper limb and microsurgery, University Malaya, Kuala Lumpur, Malaysia attended Bukit Nanas Convent, Kuala Lumpur, graduated from the Royal Free Hospital School of Medicine, University of London. She later worked under (now Emeritus) Professor Robert W.H. Pho in Singapore to be trained as a hand surgeon in 1988. After returning from Singapore, she formally began the Hand and Microsurgery Clinic at UH in 1993. A separate team began attending to hand injuries and emergencies around the clock providing full time services for many difficult cases to treat including mangled limbs and amputations. At that time, this specialised service was only provided at the University of Malaya Medical Centre.

She was Head of the Department of Orthopaedic Surgery from 1999 to 2012 and during her term, the number of staff increased to cover all major sub-specialities with considerable research output. The clinical Masters in Orthopaedic Surgery (UM) has grown, and the department now runs more than 12 courses a year.

She was a member of the first Conjoined Board of Orthopaedics and was the Chairman in 2006/2007. She was a member of the Orthopaedic Speciality Credentialling Committee of the Malaysian Medical Council and is an examiner for the sub-speciality fellowships in Orthopaedic Surgery for the Ministry of Health, Malaysia. She was an instructor for the Basic Surgical Skills Course, Royal College of Surgeons, Edinburgh for 6 years. She is a fellow of the Academy of Medicine and College of Surgeons of Malaysian.

She has written numerous papers in local and international journals, and she has published several chapters in books. She is a reviewer for several local, regional and international journals. She has supervised several students for the Masters of Orthopaedic Surgery, Masters and PhDs in research Bioengineering, Medical Science and General Surgery.

Her research interest is obstetrical and traumatic brachial plexus injuries, congenital hand problems, tetraplegic hands, mini external fixators, wound healing and advanced glycation end products. She has won several medals locally and abroad for inventions and has two patent applications. She was the Malaysian Orthopaedic Association President 2006/2007. She was a founder member of the Malaysian Society for Surgery of the Hand and was President for over 6 years. She represented Malaysia internationally in both capacities.

Her mother Elinah, is a Pianist and Musician, her father Tunku Ahmad, an accountant and corporate figure and her husband Zulkifli trained as an Architect and is a property developer and artist. They are blessed with two lively children Nur Aishah and Yaqub. In 2003 she was awarded the Dato' Setia diRaja Kedah by his Royal Highness the Sultan of Kedah.

"HANDS ON HIPS – THE WHY AND WHEREFORE"

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"Hands on Hips – the why and wherefore"

'Inaugural' can be defined as a marking of the beginning of a new venture or series, or an address, as of a president, at the beginning of a term of office. The aims and the role of the inaugural professorial lecture have been set out by other Universities worldwide. This type of lecture "or an equivalent appropriate public event" was listed as a duty of a professor "to introduce the new professor to the University community and demonstrate the scope of his or her expected contribution to the University and its stakeholders". It must be given soon after the appointment of a professor. I should apologise for my tardiness and urge my newly appointed colleagues to uphold this tradition more closely.

Inaugural professorial lectures are also given to promote and to celebrate the academic reputation of professorial staff. It is stated that the lecturers "make an important contribution to the University's role within the wider community by providing a public forum for leading research and enhancing (the University)'s role as a critic and conscience of society". In a nutshell, the event should 1) be a public celebration; 2) promote the academic reputation of the staff and scope of contribution to the university; 3) be a public forum for leading research; and 4) enhance the university's role as a critic and conscience of society.

The first aim would be met by the invitation list, and presence of one or two representatives of the top management. I should make it as entertaining as possible, too. The rest of my lecture should cover the last three aims. Hence my talk is entitled "Hands on Hip – the why and wherefore". Hands are my passion and profession; hip represents orthopaedics, the subject that I teach, and the profession of my department staff and close colleagues. 'Hands on hips' can represent a gesture of display, where we show what we have done and what we are able to do in the future, or a gesture demanding answers as a critic of society. The phrase 'Why and wherefore' represents research and learning.

Orthopaedic surgery is a wonderfully simple craft. It involves the musculoskeletal system; the muscles, bones and joints that support the body and enable it to propel itself and manipulate the environment around to fulfill our needs. In many ways it is a visible part of us, it displays to the rest of the world the way we look, the way we stand and carry ourselves in all our daily activities. It is however, primarily functional. The hand in particular is very beautiful and is built to be used both in very fine and very forceful activities.

In Malaysia, in Hand Surgery and in Orthopaedic Surgery, trauma is a big challenge. Orthopaedic trauma is so common and well-practiced that our masters' students are very good at managing and performing surgery for trauma. It is the bread and butter of Orthopaedics and it is the biggest part of hand surgery. The rest of the world comes over to learn trauma surgery from us and our Hand Unit has had two fellows from U.K. who came over to work with us to learn about how we treat many conditions but to a large extent they came to practice and improve their trauma skills. We were told by the first fellow that a paper we were reviewing from UK about replanting amputations would have seen a tenth of what we see over here and our own experience was more instructive than that paper. They learned a lot about how to deal with trauma and it improved their confidence to deal with many other conditions.

In Orthopaedic Surgery the reason probably comes on two wheels, the motorbike. In general, road traffic accidents are taking up a huge amount of time, and resources and with reduction in trauma many less urgent problems would be better dealt with. How to do this - some suggestions are to reduce the speed limit and improve policing, and improve public transport. Perhaps more studies need to be done to tell the government what steps would work. Prevention is the role of policymakers and primary care physicians, we can collaborate in research, but Orthopaedic surgeons only deal with injuries when they happen.

Emergency hand surgery is also caused, to a certain extent by motor vehicle accidents but many other incidents occur at the workplace because of ineffective workplace safety measures and still others are created entirely on purpose in fights, robberies and attacks. To our hand team, an amputated hand is a common occurrence. Slash wounds from robberies require perhaps two to three surgical reconstructions to get a reasonable looking and functioning hand. The parang is a weapon of choice it seems.

Whenever society becomes violent or unhappy, we see the result first and most dramatically. We are all happy with effective gun control, otherwise, we would see far more devastating injuries and perhaps many would not live to reach us. We desperately require better policy and a solution to drug addiction. I am sure this is both a cause and effect of the violence that is escalating in our country. Overcrowding of living conditions and poverty must also be a cause, as well as some interracial tension. We all hope policy makers and all others can work towards racial harmony and stability in our culturally rich and diverse country and also try to solve the poverty and poor living conditions of certain segments of society.

Another devastating injury that is caused by road traffic accidents is brachial plexus injuries, where the whole network of nerves giving feeling and movement to the upper limb is injured. We see this injury in two spectrums. One of which is in adults, usually young men thrown off their motorbikes. Our study of the incidence of these injuries shows that they almost exclusively occur in young male motorcyclists. We found in a multicentre trial the incidence of these injuries is 0.27 per 1000 trauma cases presenting to casualty. We do operate on these patients and it is an exhausting problem with long and difficult surgery and not dramatic results. This is a situation where we say that "a little, to a man with nothing, is a lot". Prevention would most certainly be a better idea. Again this leads us to the road traffic conditions.

The other spectrum of this problem is birth injury to infants passing through the birth canal. The shoulder may be too big to pass through the canal smoothly and the neck stretched as the head is pushed away from the shoulder. The child's nerves leading to the upper limb are stretched badly and one limb is paralysed. The child may recover partially, fully or not at all. The general incidence is known to be 1 to 8 per 1000 live births and it is not reducing even in developed countries where obstetrical care is getting better. Thus, many have hypothesized that something happens before the journey through the canal, which is in the womb of the mother. I think it may be very useful to look into the amount of force that is required to injure those nerves and to think about how to detect earlier that this may be a problem. This could be the starting point of good research. In fact research is the bane of many, and again done begrudgingly by many University doctors just to get their promotions. The problem is that people need time to contemplate. In the present time, when our country is becoming more sophisticated and we have some inclination to participate in cutting edge medical research, we have become so busy that many do not have the time to sit and think of what really needs to be discovered. We should probably try to do activities that clear our mind, more frequently.

In general, Orthopaedic Surgeons are not known for their fundamental research, which is research on the basics of what is happening in the body, at the cellular or tissue level. Personally, my first exposure to fundamental research was during a time when I was carrying out surgery on arteries and veins of patients with kidney failure. I was required to remove a bit of artery and patch a vein over the hole with microsurgical sutures. Truly the arteries of these patients could range from a smooth wall to a wall full of chalky residue and hard as a piece of cardboard. I wondered what could be the differences between the two, and I thought it was a shame that I was throwing the extra pieces of artery away when we could find out something about the wall. So after discussion with a colleague, Prof S.Y. Tan, he explained that when exposed to very high levels of sugar in the blood, the walls of the arteries underwent changes caused by what we called advanced glycation end products. Prof Tan, Prof Umah Kuppusamy, Prof Mahmood Ameen and I decided to study the Advanced Glycation End products, also known as AGE, and antioxidants in the wall of arteries and veins and compare them with vessels of normal people. This led to a grant and several papers, research assistants and supervision of a master's candidate. We found that kidney failure is worse than diabetes for arteries, and were the first to show that veins may reflect the complication better than arteries. Those findings were very useful but not earth-shattering, and that is the problem with fundamental research. It is expensive, laborious and each piece of research may not give dramatic results especially the research with less funding. However, each small piece of the truth adds up and finally be of use to mankind. This journey makes it discouraging for doctors, who are so used to making people better with surgery or medication within hours, days or weeks.

Despite the present research culture, the fundamental research of the department of orthopaedic surgery is advancing well as we have many enthusiastic and clever doctors interested in tissue engineering. This is the technique of taking cells from people, manipulating and growing them and then in their more useful form, replacing them. We are going into this in a big way, for cartilage and tendon. Recently we discussed the importance of general public awareness of research. Perhaps the general public is generally aware of the importance of cancer research, but not of other research. We need them on our side not only to help with funding but also to give us moral support.

Clinical research is no longer the luxury of other countries. Malaysians need to tell the world how we are doing in our practice, what we are doing differently from others and how these new ideas are working. The practice of attending international conferences but not participating is getting less common. Then, we used see many Malaysians at a conference but not to see their names in the programme or to hear their work. Now at last we can see some Malaysians taking an active role. It goes without saying that a University or Ministry of Health would not support a doctor to go for a conference without proof that they have had their paper or poster accepted. So who is footing the bill of all these doctors flying around the world? That is where "the industry" (selling medical equipment, pharmaceuticals and implants) is literally spoiling us, being generous but removing the drive to record and present

our work. Overseas, even a very junior doctor going to a conference would be asked to present something simple, like an interesting case. This is how they cut their teeth and get into research very early on.

I believe the industry practices differently overseas compared with here in the Far East. I don't think they are allowed to hold dialogues for European doctors in another country where the whole group of doctors could have met in their own country and the topic of discussion centres around a product of the company paying for the whole 'dialogue'. Neither are they allowed to select and sponsor certain doctors to overseas conferences. The departments or institutions decide who has performed well enough to deserve a sponsorship. The Malaysian Orthopaedic Association has several times been asked to legitimize an award where the recipient has been selected earlier by the Company. I am glad to say that the MOA did not agree.

I think it is long overdue for the companies to treat us the same way they treat our colleagues overseas, to stop spoiling us and allow us all to grow up. I must say at this point, that this does not by any means apply to all companies, the majority of whom are just trying to introduce their products and make sure we know the benefits and use them whenever they are indicated. We are grateful for the representatives who educate us and also help to run courses that educate many not only on their products but on important topics and techniques that can apply in general.

Dato Dr Mahmood Merican and Datin Ragayah are far sighted enough to realize the value of research and learning. With their help and with Prof Dato Amin Jalaluddin aboard, and a lot of valuable advice from Dato' Toh Puan Dr Aishah Ong, the Yayasan Ortopedik was formed, to help our department achieve our goals in research, service and training. The University has approved the building of NOCERAL (National Orthopaedic Centre of Excellence in Research and Learning) and work has begun to build this complex containing a GMP accredited lab, bone and tissue bank, and three other laboratories and audiovisual and training facilities. Thanks to my husband and Mr Huang Ying How from AKP architects who did the elegant conceptual designs.

Orthopaedics is very special in that there are many devices that can aid us to help patients in wards, at home, and in the operating theatre. We are also fortunate that we do not have to go far for a better device that may help in our work. Several devices have been invented by our own department staff. The finger fixator created by our team with Prof Dato' Goh Sing Yau, a Bioengineer/ Mechanical Engineer has been used on several patients with finger contractures. It was displayed in Geneva and won a silver medal. We have since worked with Prof Nor Azwan and improve the appearance and made it easier to use. This device will be used in patients very soon. We have every hope that it will be even more successful than its earlier prototype.

Our finger fixator will be used in victims of burns and crush injuries, mainly workers in manufacturing industries. Many of these patients are foreigners. It is very sad for our teams to see these patients, some of whom have only started to work for a few months when the injury crushed their goals and dreams. Not only can they not earn money for their relatives back home but they also are going back with reduced function and a deformity. Despite our best efforts we can only work with what we are given. We had a study looking at occupational-related injury or hazards. We noted that the lack of training before starting of a job was the main reason for these injuries. Our study also showed that many of these workers removed protective guards on the machines so they could insert their hands into the machines to get the job done faster. This study was presented and published in the proceedings of the yearly Malaysian conference on Injuries at the workplace. We hope it made a difference. The Social Security Organization of Malaysia or SOCSO and the National Institute of Occupational Safety and Health (NIOSH) are very concerned. Courses to estimate impairment for the purposes of accurate compensation are run by NIOSH several times a year. These organizations should probably sponsor further research that would benefit all workers. However these organizations only cover Malaysian workers when they are injured and the insurance covering foreigners seems inadequate to us. We hope that the conditions for workers as a whole will improve and that these injuries will soon be something belonging to the past. Then we can start using our new device for fingers that are bent due to disease, not injury.

Hand injuries are not the only problems we see as Orthopaedic surgeons, but all kinds of fractures and soft tissue injury and even sometimes paralysis after a fall at a construction site. These injuries occur to Malaysian as well as foreign workers and this shows that the standards of safety should be looked into. My husband's colleague told us that in Australia, if one fatality occurred on a building site, the side is closed down for investigation. I'm not sure whether this happens here, especially with foreign workers.

These foreign workers are far poorer than our Malaysian patients but pay a higher rate for treatment. Although some kind hearted employers do pay for treatment and help them out, it is all too easy for an employer to rid himself of these workers and ship them off home with minimal financial support. I must also say that we get a great many foreigners also who injure themselves trying to prevent themselves from being arrested for illegal entry or for taking part in illegal activities.

Our team not only collaborates with the engineers but also work closely with oral and maxillofacial Surgeons. In fact my first publication was describing the replacement of the mandible (or jawbone) with the fibula a bone in the leg, using microsurgical techniques. Since then we have carried out about 40 more such cases. This is usually done because tumour affects the jaw. A colleague from South Africa informed me that the main indication for these replacements at home was gunshot wounds. Again I am grateful for the strict gun control laws preventing this here. We tried several techniques over the years. The Fibula is a

straight bone and thus requires to be cut in several places in order to conform to the shape of the jaw. This can be done without damaging the vessel supplying the blood to the whole jaw. The bone can later be used as a base to implant teeth.

We as the academic staff of the university and consultants of the hospital provide links to the various professional societies of which we are members. The Malaysian Orthopaedic Association has grown from a small fellowship of surgeons to a wealthy and healthy society promoting fellowship and the advancement of skills and knowledge amongst its members locally and abroad.

The hand society on the other hand or its correct name to conform to international societies is the Malaysian Society for Surgery of the Hand (MSSH) is a work in progress. It is still very small but recently has grown probably to a critical mass to a point where it is able to help its members to progress. One or two active members have been keeping it alive all these years but last year with the addition of several younger members and the support of the stalwarts, the society continues to have meetings to discuss cases and conditions at members residences, from Klang Valley to Kuantan and to Kota Bharu and beyond. Another turning point was when we went to Hong Kong to represent the society this year. As the President, I was proud to observe Malaysians presenting a handful of papers and posters. We were also offered and agreed to host an International Conference-the Asia Pacific Federation meeting in 2014. This is really a turning point where the Society can collect funds and also become recognised internationally. I hope very much that we will have in the near future many more hand surgeons in Malaysia to give good care to our people and to contribute more to international thinking and consensus.

At the moment, if you have a hand injury, it is a hit and miss situation whether you will land up with someone who is experienced enough and knowledgeable enough to give you good care. The hand and Microsurgery service providers are overwhelmed. I am proud to say we are actually a very active centre. Hospital Kuantan, Hospital Selayang, Hospital Kuala Lumpur, the University Hospitals such as our own Pusat Perubatan University Malaya, Pusat Perubatan University Kebangsaan and Hospital Universiti Sains in Kota Bharu are doing good work, but there is a stark need for more hand and microsurgical services. I must urge the Ministry of health to train more of us. To many young orthopaedic surgeons who are trying desperately to run a good hand service, I must thank you on behalf of our patients because it is a thankless task, especially when you are not credentialed.

I would like to end on a lighter note. A sense of humour can salvage even the direst situations. At a recent conference in the USA, I listened to a wise and contented old orthopaedic surgeon who said “Always laugh with your patients (not at them)’. This, I had always done, but after the advice I decided to make a conscious effort to listen to patient’s stories and laugh with them. This way, I enjoy my clinics and teaching my students as well. Thanks to University Malaya and University Malaya Medical Centre for giving me the amazing opportunity for me to do what I do. Thank you all for your attention.

The text was presented in an inaugural lecture by Professor Dato’ Dr Tunku Sara Tunku Ahmad in Faculty of Medicine, University of Malaya on the 21st April 2008.

IATROGENIC ('CLINICIAN-INDUCED') DAMAGE INCURRED BY HUMAN SPERM DURING INFERTILITY TREATMENT: POSTGRADUATE RESEARCH AND COLLABORATIVE DEVELOPMENTS BETWEEN THE UNIVERSITIES OF MALAYA AND OXFORD

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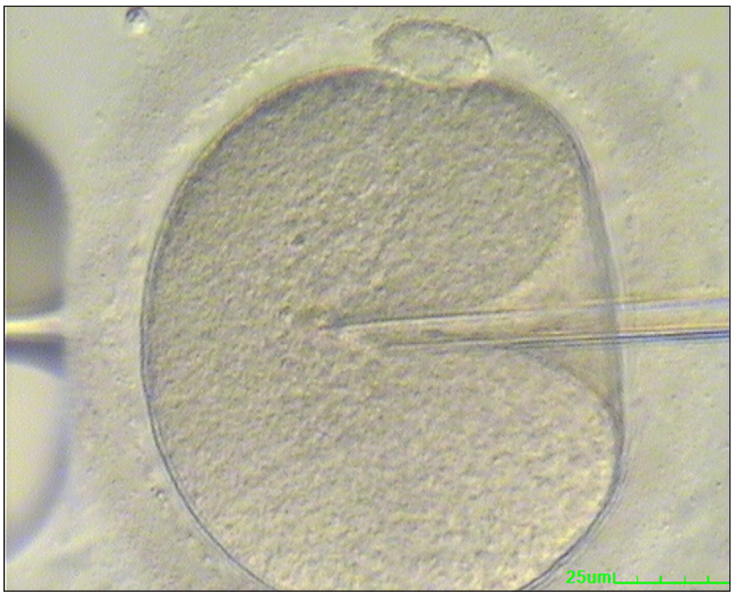

Intracytoplasmic sperm injection (ICSI) in which a single human sperm is directly microinjected into a human oocyte. The individual sperm can be seen towards the sharp end of the glass micropipette about to be transferred into the oocyte cytoplasm. Image courtesy of Tracey Griffiths (Oxford Fertility Unit)

ABSTRACT

Assisted Reproductive Technology (ART) is a suite of laboratory techniques designed to rescue infertile phenotypes. While ART has led to the birth of 5 million ART babies worldwide, success rates rarely exceed 40%. One potential factor for this could be iatrogenic ('clinician-induced') damage to critical sperm proteins, such as phospholipase C zeta (PLCζ) and protamine, which are fundamental for oocyte activation and sperm DNA integrity, respectively. This report describes how we have begun to investigate the adverse effects of ART techniques upon these key sperm proteins. We also describe the pathway taken by Miss Suseela Yelumalai to acquire a scholarship from the Malaysian Government and her postgraduate experience at the University of Oxford. We introduce the facilities and learning opportunities available at the Institute of Reproductive Sciences (IRS) which houses Dr Kevin Coward's research laboratory, and finally, highlight the potential for collaborative development between the Universities of Oxford and Malaya.

Keywords: *Assisted reproductive technology, iatrogenic damage, phospholipase c zeta, protamine*

Introduction

The prevalence of human infertility is growing rapidly and is currently estimated to affect at least 1 in 4 couples (1). Such worrying statistics have led to the rapid expansion of Assisted Reproductive Technology (ART), a sophisticated suite of techniques used to rescue human infertility under controlled laboratory environments. Thus far, ART has resulted in the birth of over 5 million babies worldwide (2) and continues to attract significant financial investment. Techniques deployed in the ART laboratory are becoming increasingly more sophisticated and involve the treatment and manipulation of male and female gametes under artificial conditions. One pioneering technique, for example, is intracytoplasmic sperm injection (ICSI), in which a single pre-selected sperm is microinjected directly into an oocyte. However, for a variety of factors, the success rate of ART rarely exceeds 40% (3), meaning that in most cases, ART is likely to fail. It is therefore imperative that scientific research strives to identify the causative factors involved such that patients continue to be provided with the best levels of clinical care. The causes of poor success in ART are highly likely to be multi-factorial in nature, and can be generally attributed to factors related to the sperm, oocyte, laboratory environment, and the technical ability of the clinical embryologists involved. Above all, ART attempts to recreate a very specialized interactive encounter between two very specialized haploid cells (the sperm and oocyte) in an artificial environment in such a manner that the oocyte is fertilized and activated to begin dividing into an embryo. Protecting the health and functional ability of the two gametes involved, and recreating the environmental conditions in which the gametes would ordinarily meet, are particularly difficult, and likely to be a major factor underlying poor success rates.

However, one particular factor that has only recently drawn attention from the research community is the increasing risk of iatrogenic ('clinician-induced') damage being inadvertently caused to gametes during ART procedures. Such damage may, for example, be caused by washing, selection, or manipulative procedures in the laboratory environment, and induce structural or functional deficiencies in sperm or oocytes (4). Consequently, identifying which techniques may be at fault, and determining how iatrogenic damage arises and manifests, is of great importance. The Coward Laboratory at the Nuffield Department of Obstetrics and Gynaecology, University of Oxford, has been investigating such issues since 2010, particularly with respect to how ART techniques may influence critical sperm proteins that play a fundamental role in sperm structure and function. In 2011, the Coward Laboratory published disturbing data showing that cryopreservation, a routine technique used in ART to store human sperm, could cause significant reductions in the levels of phospholipase C zeta (PLCζ), a protein that is responsible for activating the oocyte at fertilization (5). This implies that human sperm that have undergone freeze-thaw cycles may exhibit deficiency in their ability to induce oocyte activation (3). Current work in the laboratory seeks to investigate the effects of other routine ART techniques used to treat, select, or manipulate human sperm, upon key sperm proteins such as PLCζ, and more recently, the protamine family, which plays a fundamental role in maintaining DNA integrity in the sperm nucleus (6). Such research should lead to the modification or replacement of techniques and equipment, or lead to changes in environmental laboratory conditions, such that patients are provided with the best chances of success.

This article describes firstly, how Miss Suseela Yelumalai secured funding from Malaysian resources to join the Coward Laboratory in 2011 to take part in this pioneering field of research, and secondly, how her return to the University of Malaya in 2014 should result in the initiation of a long-lasting and fruitful research collaboration between the Universities of Oxford and Malaya.

The route from Malaysia to the University of Oxford

Miss Suseela Yelumalai graduated in 2011 with a Master's degree in Medical Sciences from the Department of Obstetrics and Gynaecology, University of Malaya, Malaysia, under the supervision of Professor Siti Zawiah Omar. In testament to her academic performance and motivation, and with the welcome support of the Vice Chancellor of the University of Malaya, Tan Sri Ghauth Jasmon, Suseela was offered two prestigious scholarships for academic study overseas leading to the award of a PhD. Suseela secured funding from the Bright Sparks scheme at the University of Malaya, and received a Skim Latihan Akademik Institusi Pengajian Tinggi (SLAI) Fellowship from the Ministry of Higher Education (MOHE) in Malaysia. After many hours searching the internet and online scientific literature databases, Suseela became increasingly drawn to the work of Dr Kevin Coward at the University of Oxford, and approached him directly by email to enquire about the possibility of registering for a postgraduate research degree under his supervision. Following numerous emails and an online interview via Skype, Suseela was offered the chance of joining Dr Coward's Laboratory for a three year period leading to the eventual award of a PhD.

Suseela joined Dr Coward's team in November 2011 and received significant help and support to help her acclimatize into her new social and academic environment. Like Dr Coward, Suseela is affiliated to the Nuffield Department of Obstetrics and Gynaecology (NDOG), one of the largest academic Obstetrics and Gynaecology departments in the UK (www.obs-gyn.ox.ac.uk). She will shortly begin her third and final year of study and has enjoyed her time in Oxford immensely. Her research focusses upon how iatrogenic damage incurred during routine ART may influence the abundance, expression and degradation of PLCζ and protamine, sperm proteins that play a critical role in oocyte activation and DNA integrity, respectively. She has already published various aspects of her work (4,7, 8) and is preparing several other research papers for

publication. She has also presented her work at a variety of national and international conferences, and will shortly be delivering an oral presentation at the annual meeting of the American Society for Reproductive Medicine in Boston, USA. Suseela has also been selected as the NDOG student representative for the Joint Consultative Committee for the Medical Sciences Division (which oversees issues and problems associated with postgraduate taught and research degrees), and the NDOG Athena Swann working group (which promotes equality and enhanced working environments in academia). In addition, she has been chosen by the NDOG to give a short talk of her experiences as an Oxford PhD student to an audience of over 200 incoming postgraduate students at the Medical Sciences Division 'Red Carpet' Event in October 2013.

The Coward Laboratory

Dr Kevin Coward graduated from the University of Stirling in Scotland with a B.Sc. (Hons) in Biological Science and a PhD in Reproductive Physiology and Endocrinology. He held Post-Doctoral Fellowships at Brunel University, Queen Mary University London, Imperial College London, and University College London. In 2002, he joined the Department of Pharmacology, University of Oxford, as a Senior Research Fellow, to investigate oocyte activation and calcium signaling at fertilization. In 2008, he moved to the NDOG as Director of the new M.Sc. in Clinical Embryology (a course which he designed and implemented; www.obs-gyn.ox.ac.uk/MSc), and as a Principal Investigator (http://www.obs-gyn.ox.ac.uk/research/kevin-coward/). He is also a Fellow of the Higher Education Academy, an organization devoted to excellence in teaching and learning. His research focuses upon oocyte activation, male factor infertility, iatrogenic damage incurred during ART, and the development of nanoparticle-mediated delivery systems to deliver research tools and clinical agents to mammalian gametes and embryos. He has published over 70 peer-reviewed articles in international journals and contributed over 70 abstracts, posters, and oral presentations to scientific conferences. In less than five years of forming his own independent research group, he has published 32 papers. The Coward Laboratory is a friendly and highly motivated research group, managed on a day-to-day basis by Mrs Celine Jones, who has worked in the NDOG for almost nine years. Celine joined the Coward Laboratory in 2008 and is jointly responsible for the MSc in Clinical Embryology. Kevin and Celine take great pride in their enthusiastic and motivated approach to teaching and research. They were awarded a Major Educator Teaching Excellence Award in 2012 for the development of the MSc in Clinical Embryology, and an OxTalent Award in 2013 for their innovative use of information technology in postgraduate teaching. The Coward Laboratory has also won several academic prizes for their research. At the time of writing, the Coward Laboratory is composed of nine personnel: Kevin, Celine, two PhD students, four MSc students, and an undergraduate medical student.

The working environment: The Institute of Reproductive Sciences

The efficiency and quality of the research and teaching carried out by the Coward Laboratory relies heavily upon the Institute of Reproductive Sciences (IRS), within which the Laboratory is housed. Opening in 2009, the IRS represents a collaborative venture between the University of Oxford (NDOG), the Oxford Fertility Unit (OFU), and Reprogenetics, UK. Collectively, this partnership creates a unique environment in which clinical diagnostics, treatment, genetic diagnosis, clinical and basic research, and postgraduate education, all occur under the one roof. The IRS provides the Coward Laboratory with state-of-the-art teaching and research facilities (**Figure 1**), fuelled by research collaboration with OFU clinicians, embryologists, and nurses.

The Oxford experience

Since her arrival in Oxford, Suseela has been introduced to a wealth of scientific techniques in reproductive science and ART, including the ethical use of human semen samples. For example, semen analysis is a routine protocol in ART units. In the Coward Laboratory, we use Computer Assisted Sperm Analysis to investigate basic sperm parameters such as progressive motility, concentration and sperm velocity distribution. Training was provided in the use of this sophisticated system for the analysis of human, mouse, boar and rat sperm. Other key techniques include sperm washing and selection techniques, cryopreservation, sperm DNA fragmentation assessment, and immunofluorescent determination of target protein expression and localization in sperm samples by fluorescent and confocal microscopy (**Figure 2**). Many other techniques are available within the Coward Laboratory including recombinant DNA technology, polymerase chain reaction (PCR), recombinant protein expression and purification, bioinformatics, nanotechnology, gamete micromanipulation, and the use of infra-red laser to biopsy embryos for genetic assessment. While focusing predominantly upon human samples acquired from the OFU and other overseas collaborators, the Laboratory also utilizes several animal models including the mouse, boar, rat, cow, and zebrafish, to inform both their research and teaching obligations. A key role of the Laboratory is to help diagnose cases of oocyte activation deficiency in infertile males attending the OFU. As part of her doctoral studies, Suseela carries out numerous research investigations upon sperm samples from infertile patients to investigate levels and localization patterns of PLCζ, and in doing so, can provide OFU clinicians with valuable information relating to the oocyte activation ability of such sperm. By virtue of her research, Suseela already plays a key role in pioneering attempts to diagnose and treat oocyte activation deficiency in humans, a condition that affects approximately 1200 cases annually in the UK alone. Her broad training in the biochemical and molecular techniques used to study sperm function in humans and a variety of animal models, and in the treatment, selection

Figure 1: Images of (a) the Institute of Reproductive Sciences (IRS), (b) the postgraduate study room within the IRS, (c) the teaching laboratory for the MSc in Clinical Embryology, (d) the micromanipulation suite, and (e)/(f) students performing micromanipulation procedures. Image courtesy of Matt Lodge.

and manipulation techniques used in ART, creates a solid foundation from which to build an independent career upon her return to Malaysia. After just six months in the Coward Laboratory, Suseela was awarded with a prestigious Oxford Travel grant which allowed her to spend two weeks at the University of Murcia in Spain, alongside Professor Joaquin Gadea, one of Dr Coward's collaborators. During this time, Suseela worked in a veterinary department carrying out a preliminary investigation of how the technique of *in vitro* maturation might influence the expression of key proteins in porcine and bovine oocytes that are involved in the oocyte activation mechanism.

A key goal of the Coward Laboratory is to provide their students with the necessary experience to succeed as an independent academic. Consequently, Suseela meets regularly with Dr Coward to discuss research and career development opportunities, and has supervised four under- and post-graduate research students thus far. She has also received dedicated teaching skills training via the University of Oxford, and will be delivering teaching on the MSc in Clinical Embryology over the next few months. Her approach to teaching and research has matured enormously during her time in Oxford, most notably in terms of her confidence and resourcefulness.

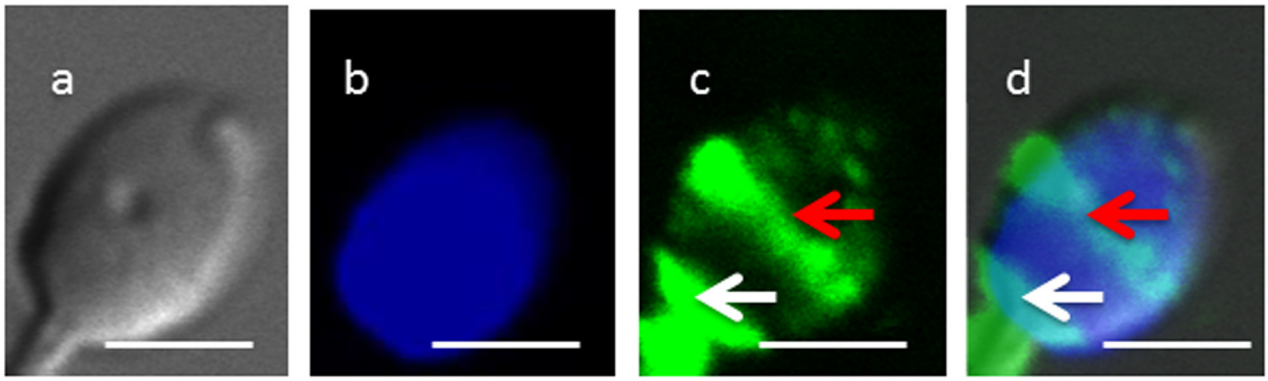

Figure 2: Confocal microscope images of fertile human sperm showing (a) differential interference contrast image, (b) nuclei, (c) phospholipase C zeta (PLCζ), the protein responsible for activing the oocyte at fertiisation, and (d) an overlay showing PLCζ localization (green) relative to the blue nucleus. Red arrow indicates one population of PLCζ in the equatorial region of the sperm head, whereas the white arrow indicates a second population in the post-acrosomal region. Scale bar represents a distance of 5μm. Image courtesy of Suseela Yelumalai.

Suseela has also received dedicated training in statistics, oral presentation skills, information technology, and academic writing skills, via the Medical Sciences Division at the University of Oxford. Such aspects of professional development are considered critical in creating a future academic capable of both carrying out informed and rigorous scientific experiments, and in communicating data and ideas to others via teaching or research presentations.

Future prospects and collaboration

The expected date for completion of Suseela's PhD is September 2014. The Ministry of Higher Education (MOHE) and University of Malaya (UM) will thus welcome Suseela's return to Malaysia in October 2014. Upon arrival, as a qualified reproductive scientist, Suseela is expected to become a Lecturer to carry out research and teach under- and post-graduates in the Faculty of Medicine. Suseela's over-riding ambition is to establish a motivated research group within the Department of Obstetrics and Gynaecology, based upon the skills and experiences she acquired during her time in the Coward Laboratory at the University of Oxford. Her fundamental goals are to pursue academic excellence and to follow Dr Coward's example in trying to inspire and motivate a friendly but highly rigorous and effective research group with clear values, principles, and integrity. Her aims are to expand upon her doctoral work by creating links with academic and clinical units both within and beyond the University of Malaya, and by maintaining active collaborative links with the Coward Laboratory in Oxford. It is hoped that such collaboration will lead to the regular exchange of students between the Universities of Malaya and Oxford, and involve key members of the Coward Laboratory spending time at the University of Malaya to contribute to teaching programs, and to initiate new research projects and other collaborative ventures (**Figure 3**). Considering the prevalence of infertility in Malaysia and the increasing number of couples seeking fertility treatment at the University of Malaya Medical Centre (UMMC), such collaborative links would enhance levels of clinical/laboratory care, thus providing an excellent opportunity for the University of Malaya to establish a ground-breaking research centre for excellence in reproductive biomedicine.

Figure 3: The University of Malaya Vice Chancellor's visit to the Malaysian High Commission, London, in May 2012. From left: Professor Dato Dr. Mohd Sofian Azirun (Dean of Faculty Science, University Malaya), Dr Kevin Coward, Tan Sri Ghauth Jasmon (Vice Chancellor of the University Malaya) Dr. Mohd Anizu Hj. Mohd Nor (Director, Education Malaysia, London), and Miss Suseela Yelumalai.

Further correspondence

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A REVIEW ON HYDROXYAPATITE-BASED SCAFFOLDS AS A POTENTIAL BONE GRAFT SUBSTITUTE FOR BONE TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

The biocompatibility and similarity of hydroxyapatite (HA) to the mineral composition of the bone has made HA a potential candidate in bone tissue engineering (BTE). Over the past few decades, its application as bone graft in combination with stem cells has gained much importance. The use of bone marrow-derived mesenchymal stromal cells (MSCs) will enhance the rate and quality of defect repair. However, application of hydroxyapatite as a material to develop a 3-dimension scaffold or carrier to support MSCs *in vitro* is still in its infant stage. This review will discuss the source, manufacturing methods and advantages of using HA scaffolds in bone tissue engineering applications.

Keywords: *hydroxyapatite, mesenchymal stem cell, porosity, sintering*

Abbreviations: BDHA, Bovine-derived Porous hydroxyapatite scaffold; BTE, bone tissue engineering; hBMSCs. human bone marrow-derived mesenchymal stromal cells; OPCs, osteoprogenitor cells; TCP, Tricalcium phosphate.

Introduction

The use of three-dimensional (3D) scaffold has been commonly accepted as an essential constituent in bone tissue engineering (1). At such, various types of scaffolds such as natural, synthetic, or a combination of both have been developed, and numerous materials have been introduced as potential materials for developing these scaffolds. Hydroxyapatite (HA), a predominant mineral component found in bones and coral, has been widely used as bone graft since the 1960s and could be a potential candidate for bone tissue engineering (2). Due to its osteoconductivity, biocompatibility and biodegradability, HA has been highly favored in surgical reconstruction of bones (3). In addition, bone implants such as titanium and titanium alloys have been coated with HA to enhance osteointegration with the host tissue (4). Later, studies on the application of HA as a scaffold for cell-based therapy have been carried out. It has been described that for the development of a successful scaffold, a 3D interconnected porous structure is necessary to allow cell attachment, proliferation and differentiation. Whilst this remains true, the use of HA in BTE has not been forthcoming owing to the high cost involved in producing the final product. This review discusses the use of HA scaffold in BTE, including my current research on the development of HA scaffold. Using a novel thermal calcination technique we demonstrated that HA in the form of porous biomaterial can be produced inexpensively with bovine bone that is readily available

in abundance (5, 6). We further tested the efficacy and biocompatibility of this material and we showed significant increased in the proliferation and differentiation of human bone marrow-derived mesenchymal stromal cells (hBMSCs) in a bovine-derived HA (BDHA) (Fig.1) scaffold that was produced using a novel thermal calcination method (6), supporting the fact that BDHA produced using our method improves MSC proliferation.

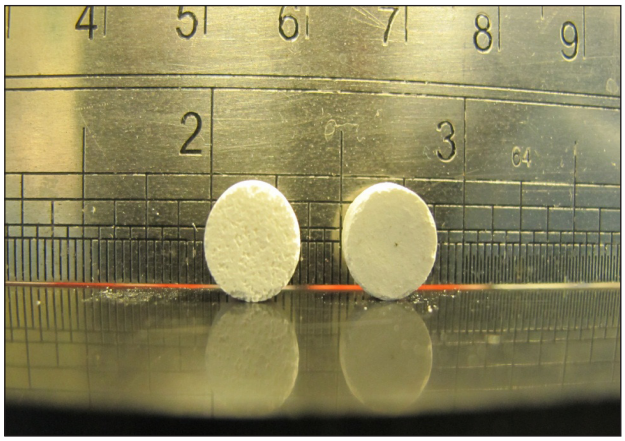

Figure 1: Bovine-derived Porous Hydroxyapatite Scaffold (BDHA)

Problems in treating critical bone defects

The management of critical-sized bone defects such as those caused by trauma and bone resection pose a substantial clinical challenge in orthopaedic surgery (7,8). Critical bone defects, being referred to a bone discontinuity that will not heal spontaneously and will require secondary intervention (9), occur mainly due to inadequate blood supply. It is possible that during trauma and surgical disruption and also instability at the fracture site, leads to the formation of delayed or non-union bones (10). These critical bone defects, which are subjected to several factors, have increased risk of non-union of the bone. These factors may include poor blood supply, infection and/or extensive soft tissue damage, bone gap or fracture comminution and inadequate fracture fixation (11). Consequently, several treatments have been used for decades to overcome these complications. Internal plate fixation and intramedullary nailing are the recommended surgical treatments for patients with these injuries. Despite sufficient stability offered by the technique, several problems such as vascular damages, soft tissue detachment and periosteal debridement have yet to be resolved (10,12). Hence, external fixators such simple or circular types have been developed based on the principles described by Ilizarov with the aim to stabilize the affected non-unions. Results of the technique employed have demonstrated good outcomes (13,14). However, distraction osteogenesis and bone transport exhibited some technical hitches, which require proper surgical training and specific set of skills (10). Reliability of internal and external fixation in treating critical bone defects were further enhanced with the use of bone graft materials. The incorporation of autografts in defect sites using internal and external fixation techniques has shown excellent vascularity and bony ingrowth within the host tissue. However, the use of autograft possesses several disadvantages such as limited availability and patient site morbidity. To overcome this issue, allograft was introduced as an alternative to autograft (14).

Tissue engineering was introduced in the early 1990s to address the limitations of tissue grafting. This technique involves the combination of cells, scaffolds and biomolecules to develop functional substitutes (16). Thereafter, various scaffolds based on calcium and phosphate compound were developed as bone-graft substitutes. Moreover, these scaffolds have further evolved by the use of cells with osteogenic potential to enhance the guided-tissue regeneration (17). The use of cells-scaffold constructs incorporation in critical bone defects appears to create similar scenarios to that of natural bone healing process.

Bone grafts as a scaffolding material

The concept of osteoconduction and osteoinduction in scaffolds

Scaffolds proposed for BTE should possess osteoconductive and osteoinductive properties. The term osteoconductive is defined as the ability of the bone to grow on the contacted surface. In another scenario where if porous scaffolds are exposed to bone tissue, the scaffolds should be able to permit cell contact and grow into the scaffolds (18). Cornel and colleagues have suggested that when osteoconductive scaffolds are placed in an osseous environment, living tissues from the host bed will migrate into the scaffold and eventually induce new bone formation and incorporation of that structure (19). This leads to a strong fixation of implanted scaffolds with the host osseous surfaces which has been clearly illustrated by Nouri and co-researchers (20). For the past few decades, autograft and allograft have been widely used in critical bone defects due to their excellent osteoconductive properties (21,22). However, owing to the limited supply of autograft and potential disease transmission of allograft, ceramics-based materials have been employed more widely. Hydroxyapatite and tri-calcium ceramic materials are the most widely used ceramic materials in orthopaedic applications. These materials are frequently used as a coating body for metal implants to enhance osteoconduction of the metal surface to the host osseous environment (23,24). On the other hand, ceramics have also been fabricated into porous 3-D scaffolds as gap filler in critical bone defects with the assistance of a fixator. Excellent host bony ingrowth has been noticed in many clinical studies. Over the years, osteoconductive ceramic scaffolds with only structural supporting network evolved into osteoinductive scaffolds which promote desirable biological events between scaffolds, cells and the host tissue.

Osteoinduction refers to the ability to induce undifferentiated multipotent mesenchymal stromal cells and osteoprogenitor cells (OPCs) to differentiate into bone forming cells by one tissue, or the product from that tissue (25). The role of osteoprogenitor cells in bone healing is apparent. At the time of injury, local multipotent mesenchymal stromal cells and osteoprogenitors cells from periosteum and endosteum stimulate paracrine and autocrine activities. The release of growth factors such as cytokines, BMP-2, TGF-β, PDGF and many recruits systemic MSCs and OPCs into the injured site. This is also known as cell homing phenomenon where cells are chemotactically attracted to the fracture site (26). These growth factors eventually cause a sequence of events which include cell proliferation and differentiation, working in concert to stimulate new bone formation. This principle has been well described. The incorporation of MSCs or OPSs and osteogenic molecules into scaffolds to improve the scaffolds from osteoconductive into osteoinductive potential has been developed over the years. In an *in vitro* preliminary study, incorporating MSCs and BMP-7 into porous hydroxyapatite scaffolds have been shown to increase cell proliferation and differentiation over the time as compared to acelullar scaffolds (27). Other studies for example, have demonstrated that human MSCs loaded HA/TCP biphasic constructs implanted subcutaneously in mice model showed the differentiation of MSCs and increased in osteocalcin expression (28).

Hydroxyapatite-based bone graft substitutes

Advantages of hydroxyapatite

Hydroxyapatite (HA) has a molecular formula of $Ca_{10}(PO_4)_6(OH)_2$. It is a member of the calcium phosphate group with 1.67 stoichiometric of Ca/P ratio (29). HA has been used widely as a bone graft substitute to treat critical bone defects since many decades ago (30,31). The primary reason for this is because HA mimics the crystalline phase of natural bone. Since 1970s, HA has been used either in blocks or as granules in multidisciplinary field such in orthopaedic and craniofacial surgery (32). During the 1970s, the real role of HA was not clearly understood. The ultimate goal in the 1970s was to just treat bone defects with autograft or allograft substitutes to enhance bone healing. Then, the outstanding outcome of HA in bone healing had encouraged researchers to gain further understanding into the role of the implant-tissue interphase between HA and host bone tissue. Since then, material-tissue interphase was extensively studied. A strong fixation between implant-tissue interphase is the first step to determine the success of an implant into the fracture site. This implant has been shown to possess bioactive surface, thus it can elicit a specific biological response at the interphase of the material, which results in the formation of a bond between the tissue and the material (33). This phenomenon is also known as biological fixation of the material to the host tissue. HA has been proven in many studies that its chemical composition can create an environment compatible for bone ingrowth (34). It also further elaborated as a class B osteoconductive material because it provides biocompatible interphase along which permits bone cells migration (35). Besides, HA is regarded as a biocompatible material because it tends to integrate well into host tissue without eliciting an immune response (36). Another advantage of HA is that it can be used as a raw material in a powder form as bone filler during small fracture or can be fabricated into 3-D scaffolds to treat large bone defects. The excellent biocompatibility of HA would suggest HA as a possible first choice in bone fracture reconstruction (37). However, despite its excellent biocompatibility, HA has inherently poor intrinsic biomechanical properties, weak tensile strength and inherent brittleness. These limit the application of HA as a high load-bearing material which necessitates the need for external or internal fixators when used clinically (37,38). Although the initial toughness of this HA scaffold is an issue, over time, the pure HA scaffold provides an ideal template for bone ingrowth, thus resulting in creeping substitution i.e. replacement of grafted HA scaffold with that of natural tissues. Furthermore, some studies have proven the notion that, the process of bone regeneration is initiated with the secretion of collagen by osteoid at a continuous rate. Over time, this material will compress and eventually improve the toughness of the newly formed bone (37,39).

Manufacturing methods of hydroxyapatite

Porous HA can be prepared either from natural or synthetic sources. Various fabrication methods are available to produce porous HA (40-42). The coralline-based porous HA can be produced by the aforementioned method. These scaffolds possess a vast range of pore size between 200 to 500 µm. In another method, starch suspension is mixed with HA powder and burned at 800°C to produce an interconnected porous scaffold. This method is known as starch consolidation where porous HA scaffolds can be produced using natural or synthetic HA. Later, gel-casting polymer sponge method was introduced by Ramay and partners. This method produced a uniform and interconnected HA scaffolds with a pore size between 200 to 400 µm (43). Another common method to produce porous HA scaffolds from synthetic HA is the slurry foaming method. This method has been employed to produce a porous scaffold with porosity around 30 to 40 % and pore size between 100 to 500 µm. In our study, a novel thermal calcination technique was used to produce BDHA scaffold (6) and the preliminary study demonstrated that the material produced using this technique not only possess the desired quality but can be produced at a lower cost, larger quantities and without the use of harmful chemical. Micro-computer tomography images of porous BDHA are shown in figure 2 A and B.

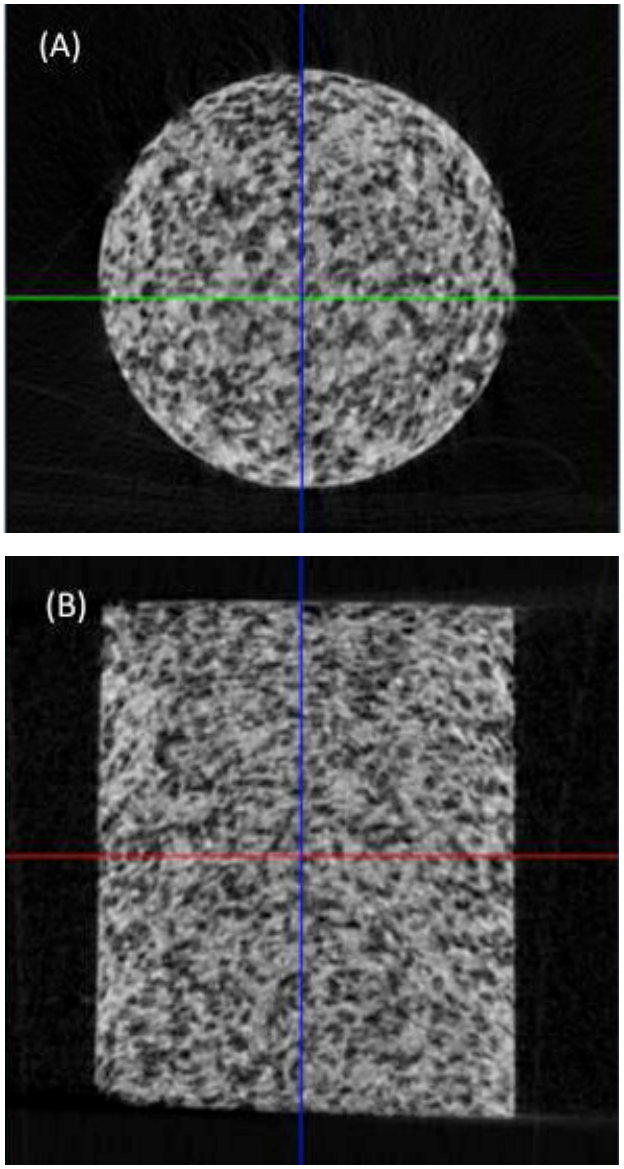

Figure 2: Micro computer tomography of BDHA, (a) Coronal view, (b) Sagittal view.

Sources of hydroxyapatite

HA can be synthesized from natural or synthetic derivatives. A common natural HA is derived from marine coral. This coral-based HA has a natural structure to the cancellous bone and the chemical composition of HA of coral is similar to that of natural bone. They can be produced via hydrothermal conversion of calcium carbonate skeleton of coral into calcium phosphate HA (44). Another source of HA is through the direct conversion of human cancellous bone into calcium phosphate HA via hydrothermal process (45). HA can also be produced from bovine bone by de-fatting continued calcination at 900°C using hydrothermal process (5).The synthetic HA can be produced from chemical reaction of calcium and phosphate elements via chemical precipitation method. Calcium nitrate and di-ammoium hydrogen phosphate salts have been precipitated from the aqueous solutions to produce pure synthetic HA powder (46). Another similar precipitation method employed by Bouyer and partners to produce synthetic HA using calcium hydroxide and orthophosphoric acid (47). Sol-gel method is another way to produce pure synthetic HA using calcium and phosphorus at molecular level (48). The production of porous scaffold using natural and synthetically-derived HA will be discussed in later sections.

Issues of HA in clinical applications

There are several issues related to HA in clinical application. In comparison to class A biomaterials such as bioactive glasses and ceramic glasses, the rate of bone bonding with HA after implantation is relatively low (49). Therefore, the recovery time of patients is fairly long. The synthetic HA bone graft substitutes fabricated using chemical process may also elicit immune reaction in the body. Cost of scaffolds manufacturing would be another issue in clinical application. Due to the exorbitant cost involved in the production techniques and expensive raw material, cost-effective manufacturing techniques have become a major issue for the use of HA in clinical applications (50).

Advances in hydroxyapatite development to enhance bone healing

The application of bone graft substitutes in critical bone defects has further evolved in tissue engineering with the advent of using cell, scaffold and various growth molecules (51). HA has always been regarded as an exclusive osteoconductive material (52). With the emergence of cell-based therapy, this has changed the paradigm of HA from being osteoconductive only into osteoinductive material. This has been achieved through the introduction of cells or growth factors into the HA scaffolds. For instance, biological properties of HA can be improved by doping small amount of elements such as growth factors, found in physiological bone (53). These constructs influence dissolution rate of apatite and have induced the proliferation of human osteoblast-like cells *in vitro*. This process may encourage osteointegration of implant to the osseous environment. Such notion has been proven via many clinical studies where implanted cell-HA construct has improved the bone healing in critical size defects (54). In our *in vitro* study, we have demonstrated that BDHA which has been loaded with MSCs showed significant increased in cell proliferation as compared to monolayer control (6).

Summary

This review paper described the potential use of HA underpinned its scaffolding or carrier properties for mesenchymal stromal cell in bone tissue engineering. Various manufacturing methods have been introduced to produce the most functional HA scaffold, yet the one that endows all the ideal quality in human bone reconstruction has not materialized. Therefore, there remain rooms for the development of this material in the advancement of bone tissue engineering. The porous BDHA scaffold

introduced in the present study provides the fundamental steps to evaluate the biological properties of the scaffold such as cell proliferation and osteogenic potential of future ceramics based scaffolds. However, *in vivo* study to demonstrate cell proliferation and osteogenic potential of scaffold is highly imperative.

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