World Journal of Stem Cells

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4252/wjsc.v7.i2.266

World J Stem Cells 2015 March 26; 7(2): 266-280
ISSN 1948-0210 (online)

REVIEW

Role of nanotopography in the development of tissue engineered 3D organs and tissues using mesenchymal stem cells

Shima Salmasi, Deepak M Kalaskar, Wai-Weng Yoon, Gordon W Blunn, Alexander M Seifalian

Shima Salmasi, Deepak M Kalaskar, Alexander M Seifalian, UCL Division of Surgery and Interventional Science, Centre for Nanotechnology and Regenerative Medicine, University College London, NW3 2PF London, United Kingdom
Wai-Weng Yoon, Royal National Orthopaedic Hospital, Stanmore, HA7 4LP Middlesex, United Kingdom
Gordon W Blunn, Institute of Orthopaedics and Musculo-Skeletal Science, University College London, WC1E 6BT London, United Kingdom
Gordon W Blunn, Institute of Orthopaedics and Musculo-Skeletal Science, Royal National Orthopaedic Hospital, Stanmore, HA7 4LP Middlesex, United Kingdom
Author contributions: All authors contributed equally to the manuscript in terms of writing the paper.
Conflict-of-interest: Authors confirm that no conflict of interest exists regarding this manuscript.

Correspondence to: Alexander M Seifalian, Professor, UCL Division of Surgery and Interventional Science, Centre for Nanotechnology and Regenerative Medicine, University College London, 9th Floor, Royal Free Campus, Rowland Hill Street, NW3 2PF London,
United Kingdom. d.kalaskar@ucl.ac.uk
Telephone: +44-020-77940500
Fax: +44-020-74726444
Received: July 29, 2014
Peer-review started: July 30, 2014
First decision: September 29, 2014
Revised: November 7, 2014
Accepted: December 3, 2014
Article in press: December 10, 2014
Published online: March 26, 2015

Abstract

Recent regenerative medicine and tissue engineering strategies (using cells, scaffolds, medical devices and gene therapy) have led to fascinating progress of translation of basic research towards clinical applications. In the past decade, great deal of research has focused on developing various three dimensional (3D) organs, such as bone, skin, liver, kidney and ear, using such strategies in order to replace or regenerate damaged organs for the purpose of maintaining or restoring organs' functions that may have been lost due to aging, accident or disease. The surface properties of a material or a device are key aspects in determining the success of the implant in biomedicine, as the majority of biological reactions in human body occur on surfaces or interfaces. Furthermore, it has been established in the literature that cell adhesion and proliferation are, to a great extent, influenced by the micro- and nano-surface characteristics of biomaterials and devices. In addition, it has been shown that the functions of stem cells, mesenchymal stem cells in particular, could be regulated through physical interaction with specific nanotopographical cues. Therefore, guided stem cell proliferation, differentiation and function are of great importance in the regeneration of 3D tissues and organs using tissue engineering strategies. This review will provide an update on the impact of nanotopography on mesenchymal stem cells for the purpose of developing laboratory-based 3D organs and tissues, as well as the most recent research and case studies on this topic.

Key words: Nanotopography; Mesenchymal stem cells; Tissue engineering; Nanotechnology; Three dimensional organs/tissues; Scaffolds

Core tip: Tissue engineering and nanotechnology are both exciting fields that have enormous potentials to revolutionise medicine as we know it today. Use of nanotechnology is an attractive and effective way to control and direct biological events at cellular levels. Nanoscale architecture plays a pivotal role directing cellular activities. Here, the use of nanotopography for the purpose of 3D organ/tissue regeneration using mesenchymal stem cells (*i.e.*, their proliferation, differentiation and function), is reviewed by investigating the most recent, innovative, and effective studies in this field.

Salmasi S, Kalaskar DM, Yoon WW, Blunn GW, Seifalian AM. Role of nanotopography in the development of tissue engineered 3D organs and tissues using mesenchymal stem cells. *World J Stem Cells* 2015; 7(2): 266-280 Available from: URL: http://www.wjgnet.com/1948-0210/full/v7/i2/266.htm DOI: http://dx.doi.org/10.4252/wjsc.v7.i2.266

INTRODUCTION

It is becoming progressively evident that with an ever increasingly older population and high costs associated with meeting the healthcare demands[1] as well as the shortage of organs and effective therapeutic methods[2], the field of medicine has to move towards cutting edge, laboratory engineered techniques and devices if, we are to avoid a drastic world-wide healthcare collapse in the near future. To this end, the fields of nanotechnology, regenerative medicine and tissue engineering (TE) are expanding at a rapid pace. Recent regenerative medicine and TE strategies (using cells, scaffolds, medical devices and gene therapy) have led to fascinating progress of translation of basic research towards clinical applications[1,3]. In the past decade, great deal of research has focused on developing various three dimensional (3D) organs, such as bone[4], skin[5], liver[6], kidney[7], and ear[8], using such strategies in order to replace or regenerate damaged organs for the purpose of maintaining or restoring organs' functions.

Human organs are responsible for various important functions of the body including, but not limited to, digesting food, serving as a barrier against infections, recognising and coordinating the body's response to its internal and external environmental changes, and providing oxygen (to be used for cellular respiration) as well as removing excess carbon dioxide. They are also responsible for maintaining homeostasis, transmission of information and generating force[3]. In cases when one or a few of the organs are severely damaged, to an extent that they are no longer capable of reconstructing or regenerating themselves, tissue engineering and nanotechnology based strategies, using previously established knowledge on cellular behaviour[9,10], could be employed to develop and construct tailored therapies, devices, or even whole organs. To this end, nanostructured, bio-inspired, or biological materials have attracted a great deal of attention as they poses unique chemical, mechanical and surface characteristics that could prove useful for organ or tissue TE.

The surface properties of a material or a device are key aspects in determining the success of the implant in biomedicine, as the majority of biological reactions in human body occur on surfaces or interfaces[11]. Furthermore, it has been established in the literature that cell adhesion and proliferation are, to a great extent, influenced by the micro- and nano-surface characteristics of biomaterials and devices[12,13]. In addition, it has been shown that the stem cells', mesenchymal stem cells (MSCs) in particular, functions can be regulated through physical interaction with specific nanotopographical cues[3,14], further indicating the importance of surface characteristics at nanometre length scale. Therefore, guided stem cell proliferation, differentiation and function are of great importance in the regeneration of 3D organs and tissues using TE strategies.

This review will provide an update on the impact of nanotopography on MSCs for the purpose of developing laboratory-based 3D organs, as well as the most recent research and case studies on this topic.

NANOFABRICATION OF 3D SCAFFOLDS WITH STEM CELLS

Previously, most research and investigations focused on growing cells in a petri dish (2D). However, in nature cells use 3D template of extracellular matrix (ECM) to shape functional tissues[15]. Micro- and nano-scale chemical and physical cues from the ECM environment control and direct various key cell behaviours including their adhesion, proliferation, migration and differentiation[16-18]. Therefore, the construction of a synthetic system that mimics the natural ECM and its component has become a field of topical interest[19].

Recent investigations have shown rapid success in TE of sophisticated and complex nanoenvironments suitable for 3D growth of stem cells for the purpose of organ and tissue regeneration[19-22]. So far, various biofabrication techniques have been developed and employed to design an ideal 3D synthetic ECM-mimetic system that resembles the architecture and mechanical properties of the natural ECM[3]. Natural or synthetic polymers are used as scaffold materials and, depending on their nature, suitable biofabrication techniques are used to create a 3D environment with nanotopographical cues that can lead to controlled and directed growth and differentiation of stem cells toward a specific tissue or organ regeneration. Numerous studies have covered the currently available fabrication techniques for natural or synthetic polymers[15,23-27]. In general, the available fabrication techniques can

Table 1 Classification of various types of nanotopography (nanofabrication) methods

Energy source	Method	Mechanism and final outcome	Processable polymers
Thermal	Replica modelling	Creating negative shape of the mold by thermal cross-linking of cavity-filled pre-polymer	Thermocurable polymers, *e.g.*, poly(dimethyl siloxane)
	Nanoimprint lithography	Creating negative shape of the mold by plastic deformation of polymer above Tg	Thermoplastic, *e.g.*, polystyrene, poly(lactic acid), and conductive polymers, *e.g.*, polyaniline and polypyrrole
	Block copolymer lithography	Creating nanoscale hole, line and lamellar structures by microphase separation of two immiscible polymers	Block copolymer, *e.g.*, polystyrene-block-poly(methyl methacrylate), styrenebutadiene-styrene
Optical	Photolithography	Depending on mask design and selective UV exposure, solubility is changed	Photo curable polymers, *e.g.*, photoresist, polyurethane-based
	E-beam lithography	Formation of arbitrary patterns using different electron beam pathways and selective irradiation of focused electron beams to change solubility	E-beam sensitive polymers, *e.g.*, polymethyl methacrylate
	Direct laser writing	Formation of arbitrary patterns by selective cross-linking of the polymer by laser irradiation	Photo-curable polymers
Chemical	Microcontact printing	Creating extruded patterns of elastomeric stamp using relative surface energy difference needed for transferring materials	Proteins and self-assembled monolayers
	Dip-pen lithography	Formation of arbitrary patterns by direct writing of molecules with a sharp tip	Self-assembled monolayers
	Salt leaching/gas foaming	Formation of a block of polymer with voids by dissolution of salt particles (salt leaching) and/or bubble formation in the polymer block (gas foaming)	Solvent soluble polymers, *e.g.*, thermoplastic and conductive ones
Electrical	Electrochemical deposition	Forming negatively shaped molds by electrochemical reduction of the polymer	Conductive polymers
	Electrospinning	Drawing a three dimensional nanofibrous mesh from the polymer solution using an electric field	Solvent soluble polymers
Physical	Capillary force lithography	Formation of partially filed negative shape of the mold by capillary rise of thermoplastic polymer above Tg	Thermoplastic and solvent soluble polymers
	Micromolding in capillaries	Creating a negative shape of the mold by capillary-driven microchannel filling	Solvent soluble polymers
	Wrinkle	Formation of random or aligned micro- or nanolines using mechanical buckling Mechanical buckling between elastic substrate and rigid film	Elastomeric polymers, *e.g.*, polydimethylsiloxane
	Crack	Formation of aligned or inter-crossing line patterns by mechanical fracturing of the stiff film adhered onto elastic substrate	Elastomeric polymers

Adapted from Kim *et al*[3].

be classified into different categories based on their energy source, *i.e.*, thermal, optical, physical, chemical or electrical (Table 1). It is beyond the scope of this paper to review the different available techniques in each of these categories, however, a review conducted by Kim *et al*[3] could be referred to for further and in detail information on this topic.

CHARACTERISTICS OF MSCs AND THEIR APPLICATION IN TE OF 3D ORGANS

An extensive number of studies have demonstrated the great potentials of using MSCs for TE approaches[28-33]. Among many advantageous characteristics of MSCs the fact that these cells can be isolated from several tissues and that they have the potential to be expanded in culture and exhibit multilineage differentiation (Figure 1) make MSCs a highly interesting stem cell source for TE and regenerative medicine research[31]. Other interesting properties of MSCs include; their ability to self-renew, modulate immune responses, and their availability (they can be obtained from a small scale aspirate of bone marrow or adipose tissues)[34-36]. Furthermore, MSCs can be isolated from adults, therefore, allogeneic transplant of these cells would eliminate raising ethical issues in regards to their use in TE and regenerative medicine[36].

Among most potential characteristics of MSCs, it is probably their ability of multilineage differentiation that is mostly exploited for TE and regenerative medicine purposes. The differentiation of MSCs is controlled by some regulatory genes and induction chemicals that lead to the specific differentiation of these progenitor cells[37,38]. In addition to growth factors and induction chemicals, various biomaterials (*i.e.*, natural and synthetic polymers) are used to provide appropriate scaffolding for the proliferation and differentiation of MSCs for the purpose of reconstruction of several hard and soft tissues and organs, such as bone, cartilage, tendons, and skin[39,40] (Figure 2).

THE ROLE OF NANOTOPOGRAPHY ON THE GROWTH AND PROLIFIRATION OF MSCS

As mentioned earlier, surface nanotopography of

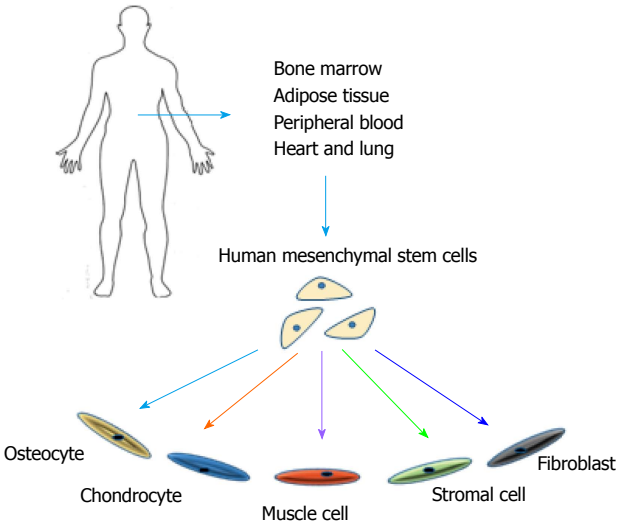

Figure 1 Potentials and sources of mesenchymal stem cells. Mesenchymal stem cells can be collected from various sources within human body and have the ability to differentiate into a variety of lineages.

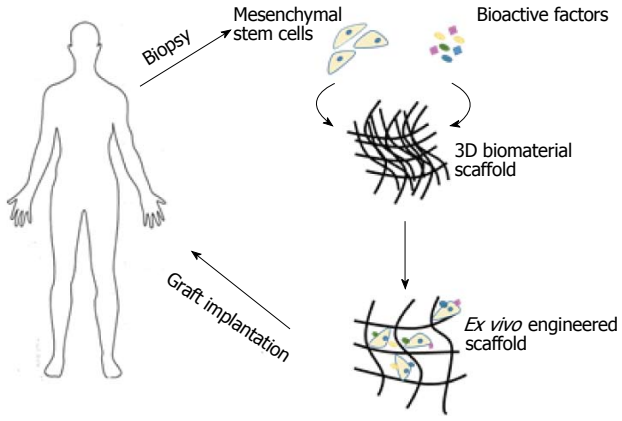

Figure 2 Overview of tissue engineering strategy of incorporating scaffolds with mesenchymal stem cells. Mesenchymal stem cells (derived directly from the patient) are expanded in the laboratory, whereby the necessary environment for their growth has been prepared. These cells are then seeded onto a scaffold and either allowed to differentiate *ex vivo* pre-implantation or the scaffold is immediately implanted.

biomaterials can evoke specific cellular responses. Materials with unique nanotopographical characteristics offer properties, similar to growth factors, which can be used to induce specific biological performances of safe and cost effective manners in the human body[41]. Previous studies show that various nanotopographical cues can potentially impact the adhesion[42,43], orientation[44], and cytoskeletal organisation[45] of MSCs as well as their self-renewal[46], proliferation and differentiation[41]. Furthermore, nanotopographical cues could influence morphology, migratory capacity, gene expression and subsequently the fate of MSCs[47,48].

It has been shown that nanofeatures including nanopits, nanogratings and nanoprotrusions have the potentials to influence the cell morphology, proliferation and differentiation of MSCs[49,50] (Figure 3). For instants, it has been shown that homogenously nanopatterned and chemically modified surfaces can have direct effect on cellular responses of MSCs, including their self-renewal abilities, control over their initial cell interactions and subsequently their cell phenotype, by creating arrays of nanodots using dip pen nanolithography[51] (Figure 4). Furthermore, differentiation and proliferation of human MSCs (hMSCs) were investigated on nanogratings of 350 nm width combined with biochemical cues such as retinoic acid, and it was shown that synthetic nanostructures can induce hMSCs to differentiate into neuronal lineage[52]. This study, conducted by Yim *et al*[52], also confirmed the significance of nanotopography as it revealed that retinoic acid alone on unpatterned surfaces did not lead to strong neuronal marker expression as it was shown on surfaces with nanogratings. Other nanopatterned structures, such as grooves, ridges, and pores as well as holes, nods, or rods are of other commonly techniques currently employed to change unpatterned surfaces for MSCs to grow on and to direct their cellular responses[49]. Such nanostructures have great applications to all areas of TE. For instance, Andersen *et al*[53] investigated adhering nanoparticles containing different small-interfering RNAs (siRNAs) into nanostructured scaffolds consisting of nanopores and reported of spatial retention of the RNAs within nanopores seeded with MSCs, which resulted in enhanced osteogenic and adipogenic differentiation of MSCs[53]. This is an exciting finding as the ability of directing a single type of differentiation plays a crucial role in developing specific 3D organs. In another study, Watari *et al*[54] used topographically-patterned substrates containing anisotropically ordered ridges and grooves to modulate osteogenic differentiation in hMSCs[54]. They reported that hMSCs cultured on 1400 or 4000 nm pitches, compared to those seeded on 400 nm pitch or planer control, exhibit better elongation

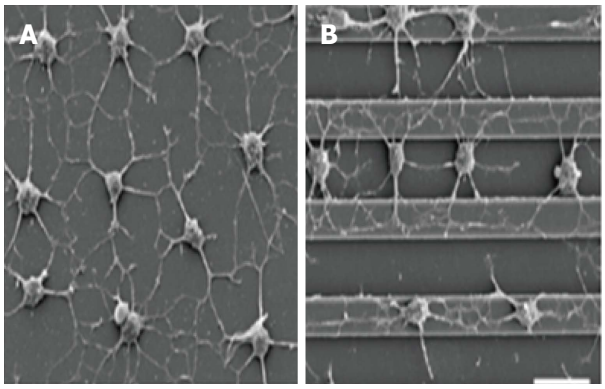

Figure 3 Comparison of different topography strategies employed to investigate the effects of anisotropic *vs* isotropic cytoskeletal tension on cultured mesenchymal stem cells. (A) nonpatterned substrates caused randomly oriented cell protrusions to be formed, while (B) alignment of elaborated processes in the direction of the grooves were induced by micropatterned surfaces, mimicking the native structure and orientation of the natural extracellular matrix proteins[52].

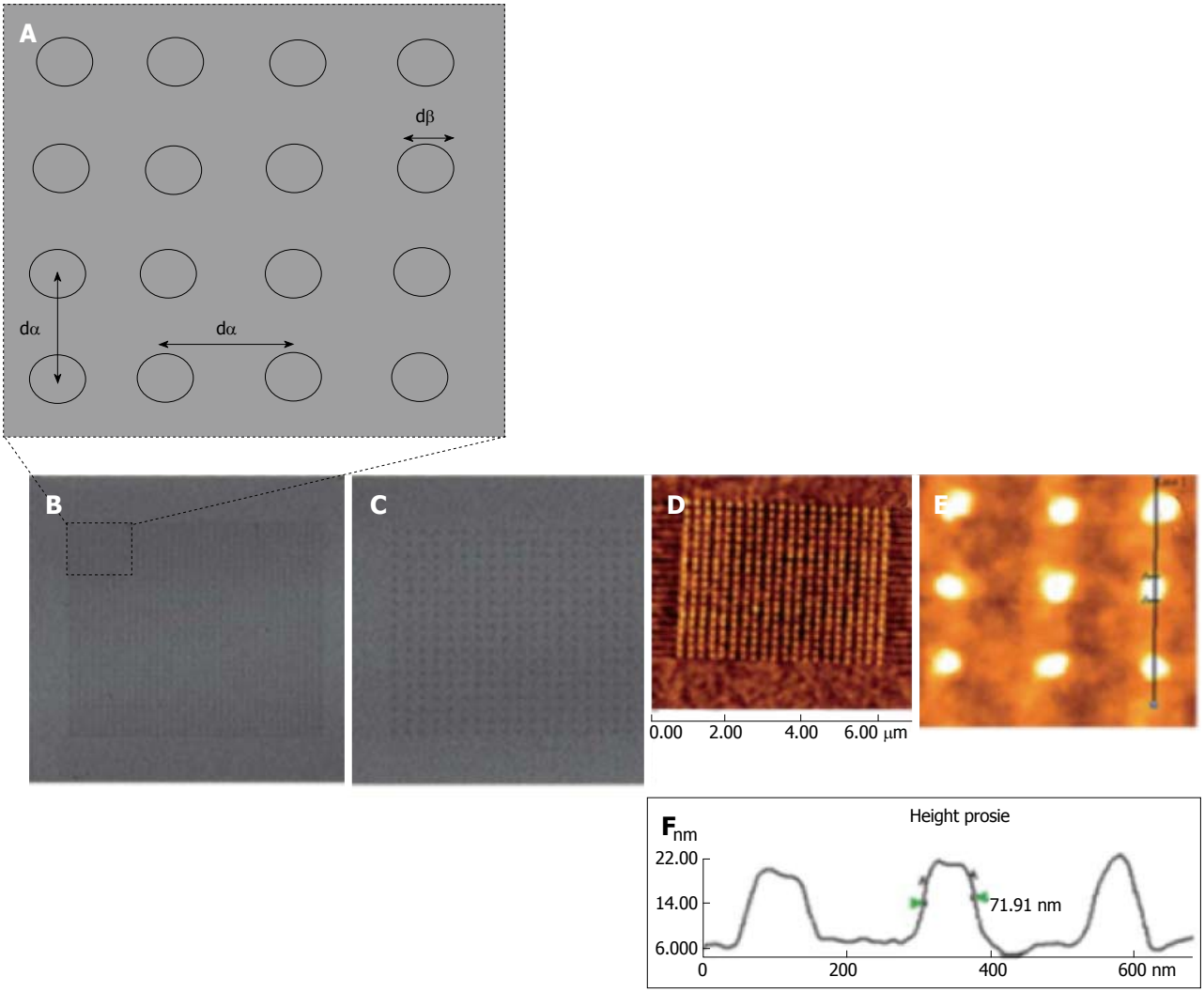

Figure 4 Nanopatterned gold surfaces examination for the effect of both the nanotopography and terminating chemical functionality. A: Nanopatterned surfaces used for mesenchymal stem cell control and differentiation exhibiting dot to dot pitch (dα) and dot diameter (dβ); B: Lateral Force Microscopy (LFM) image of small area 280 nm pitch array; C: LFM image of 140 nm pitch array; D-F: Atomic force microscopy topographical image of an alkanethiol resist array fabricated on gold surface following chemical etching. An average diameter feature (dβ) of 70 nm was shown on the cursor profile[49].

and alignment, while they showed a significant decrease in Runt-related transcription factor 2 (RUNX2) and bone gamma-carboxyglutamic acid-containing protein (BGLAP) expression. Their data also revealed that 400 nm pitch increased extracellular calcium deposition. Watari *et al*[54] concluded that specific size scale of topographic cues could directly influence the osteogenic differentiation of hMSCs both with and without osteogenic agents. This is another important finding that could enable one to manipulate and develop nanostructures that could lead to controlled and directed differentiation of stem cells for the purpose of TE of 3D organs. Very recently, the effect of topographical design, in the form of nano-pillar, nano-hole and nano-grill, on hMSCs were investigated by Wu *et al*[55] in which these nanotopographies were applied onto a polycaprolactone surface using thermal nanoimprinting. Their findings revealed that nanotopographical patterns trigger changes in the morphology and cytoskeletal structure of hMSCs. They also found that, compared to non-patterned surfaces, nano-pillar and nano-hole topography determined MSCs chondrogenesis, resulting in specific cartilage formation. Furthermore, Kilian *et al*[56] showed that geometric nanotopography cues, that increase actomyosin contractility, could influence and direct the osteogenesis of bone marrow-derived hMSCs. Such geometric cues direct and control mechanochemical signals and paracrine/autocrine factors necessary for specific differentiation of MSCs, also observed during the *in vivo* investigation of the microenvironment of the differentiated cells.

CASE STUDIES ON THE APPLICATION OF NANOTOPOGRAPHY GUIDED TE OF 3D ORGANS/TISSUES USING MSCS

Bone

Reconstruction of large bone defects caused by

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com