Journal of Science and Applications:
BioMedicine
Review

Stem cell therapy and tissue engineering approaches for pelvic organ prolapse

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Abstract: Pelvic organ prolapse (POP) is a common disorder affecting the quality of life for millions of women worldwide. Effective surgical procedures involving synthetic permanent meshes exist, but significant short- and long-term complications occur. Stem cell-based tissue engineering presents an alternative approach, which aims at repairing the anatomical components of pelvic supporting structures. However, the vagina is a complex organ with great demands of functionality, and the perfect match of scaffold, cells, trophic factors and animal model has yet to be found and tested in preclinical studies. Therefore, new research directions include the usage of bioactive materials, the application of seed cell, and the usage of various trophic factors may help facilitate integration of the new tissue. More research with detailed in vivo and long term testing is needed to ascertain the most successful and safe methods and materials for POP.

Published by www.inter-use.com. Available online May 12, Vol. 4 Iss. 3, Page 26-32.

Keywords: Stem cell, Tissue engineering, Pelvic organ prolapse, Review

1. Introduction

Pelvic organ prolapse (POP) is common condition characterized by the descent of uterus into the lower vagina or vaginal walls protrude beyond the vaginal opening. It is estimated that nearly 40% of women older than 50 years present some degree of prolapse on examination[1]. Surgery is the preferred treatment option for women who suffering from severe POP[2]. Furthermore, recurrence of POP is common when treated using ungrafted methods. Although the usage of synthetic mesh or biological grafts provides structural reinforcement to the wakened tissue of POP, complications such as foreign body reaction, excessive fibrotic response, would infections and vaginal erosion are unacceptable high, which leads to the need for surgical revision and occasionally removal of the mesh[3].

Recently, cell transplantation and stem cell-based tissue engineering are rapidly emerging as a potential strategy for tissue repair and regeneration in virtually every field of medicine[4]. Stem cells could exert a therapeutic effect via promoting the secretion of bioactive factors that have antipoptotic, antiscarring, neovascularization and immunmodulatory effects on innate tissues and direct innate stem or progenitor cells to the area of injury[5]. Multiple treatment avenues using stem cells for POP have been evaluated with animal models demonstrating their potential to restore function[5, 6]. Nonetheless, many challenges remain translate these promising results to clinical practice.

In this review, we provide a brief overview of some of the most prevalent clinical conditions that constitute pelvic organ prolapse. We review stem cell sources and their potential mechanisms of action in aiding tissue repair. We then discuss the key trials using stem cell therapy for POP, and, finally, highlight some of the challenges in translating this promising research from the bench to the bedside as well as future avenues for development.

2. Tissue engineering in urogynecology

A tissue engineering strategy with a scaffold and stem cells could be used either alone or as an adjunct to conventional surgery in the treatment of urogynecology diseases[7]. Multiple treatment avenues focusing on cell-based injection therapy for treating stress urinary incontinence have yielded promising results[8]. Local injections of stem cells have all demonstrated efficacy

in animal models of either mechanical, nerve, or external urethral sphincter injury, as demonstrated by both anatomic and functional outcomes[9]. Recently, a few clinical studies have described the different types of cells including muscle-derived stem cells, mesenchyman stromal cells or myoblasts and fibroblasts for treatment of patients with SUI, which had shown promising efficacy and safety[10, 11]. Gotoh et al assess the efficacy and safety cell therapy for 11 males SUI with a mean 1-year follow-up. Their results showed that periurethral injection of autologous ADSCs could decrease frequency and amount of incontinence, and improved quality of life without significant adverse event observed peri- or postoperatively[12]. Peters *et al* performed a prospective dose-ranging study to assess the 12-months safety and potential efficacy of autologous MDSCs in 80 female patients with SUI. Their results demonstrated injection of autologous MDSC in a wide range of doses (10, 50, 100 and 200×106) appear safe with no major treatment and a higher percentage of MDSCs presented greater efficacy[13]. Although clinical trials had showed a positive effect of cell-therapy for SUI, high-quality prospective randomized trials with more number of patients are needed in future[14].

The potential use of cell-based tissue engineering strategies to treat POP seems to be more intricate. The method of cell-therapy for SUI is unsuitable for POP because the vagina a complex organ with great demands of functionality, strength and elasticity[11]. It is known that the reconstructive surgery for hernias is closely related to that of POP and a number of tissue engineering approaches have already been explored in this field[15]. However, unlike abdominal wall repairs, one significant difference between POP repair with mesh and abdominal hernia repairs is that the pelvic meshes are generally placed adjacent to squamous epithelium rather that muscle[16]. Therefore, the complications such as mesh erosion or shrinkage with associated pelvic pain or pain with intercourse increased after POP repair with mesh[17, 18]. Tissue engineering with three sections candidate cells, scaffolds and trophic factors may solve the problem. A biodegradable scaffold, similar with the mesh used previously, not only provides a three-dimensional substrate in which cells can be delivered at high loading efficiency, grow, and form new tissue, but also provide temporary mechanical support to the weakened supportive tissues of the pelvic floor. As the scaffold gradually degraded, it will allow cells to grow and provide permanent support either directly by generating new tissue from transplanted cells or indirectly by paracrine stimulation of resident-tissue stem cells[19].

The idea of using cell-based strategies to treat POP has been very sparsely explored in preclinical experiments. However, we did not find any clinical studies that evaluated the efficacy and safety of cell-based strategies for POP by searching the databases such as Pubmed, web of science and Clinicaltrials.gov. Based on the combined experiences in these areas, the following sections focus on candidate cell types, scaffolds, and trophic factors for cell-based POP therapy.

3. Tissue engineering for POP

3.1 stem cell

3.1.1 muscle-derived-stem-cell, MDSCs

MDSCs, a possible predecessor of satellite cells, display capacities of long-term proliferation, high self-renewal, immune-privileged behavior and a superior capacity[20]. Numerous research studies indicated MDSCs could differentiate in vitro and in vivo into skeletal myotubes, osteoblasts, chondrocytes, and neural cells[20, 21]. As a stem cell source of autologous transplantation, MDSCs have several advantages because skeletal muscle is the largest organ in the body and can be obtained easily and safety. A study by Ho *et al* demonstrated MDSCs cultured on monolayer and seeded on small intestine submucosa (SIS) scaffold could differentiate into smooth muscle cells[5]. The combined of MDSCs and SIS scaffold could stimulate the vaginal tissue repair, which could have potential implications in treatment of POP in human[5]. However, we have to emphasize that the use of autologous MDSCs is time demanding, expensive, and subject to strict and increasing regulatory demands according to the current methods. Whether such an approach can be applied to treating POP remains to be clarified.

3.1.2 Fibroblasts

More recently, cells from vagina have been used primarily for the SUI, but with mitigated results and a few research groups have shown interest in the vaginal fibroblasts and their potential for the treatment of POP[22]. The fibroblastic smooth muscular tissue of the vaginal wall and its supporting tissue contain many fibroblasts, and the idea of using autologous vaginal cells for POP repair seems obvious[22]. Previous study

observed that vaginal fibroblasts exhibiting high collagen I/III ratios in cell culture also had higher proliferative rates. A study was carried out by Hung *et al* in 2010[23], in which human vaginal fibroblasts that cultured beforehand in order to amplify the number of cells were seeded on synthetic biodegradable PLGA scaffold. Then, they were implanted subcutaneously on the back of mice. Results showed that collagen almost completely disappeared at the end of a month, even though the fibroblasts were still present and formed a tissue-engineered fascia equivalent.

3.1.3 Bone-derived mesenchymal stem cells, BMSCs

Bone marrow is the first source reported to contain MSCs, which was harvested by using their adherence to glass or plastic[24]. It has been widely reported that BMSCs were capable of differentiating adipogenic, osteogenic, myogenic and chondrogenic cells. Currently, most preclinical and clinical studies on stem cell-based treatment of SUI using BMSCs have shown impressive efficacy. They demonstrated that the BMSCs have the ability to differentiate their phenotype towards smooth and striated muscle with desmin expression and α-smooth muscle actin up-regulation. However, for clinical use, bone marrow may be detrimental due to the highly invasive and painful procedures required for procurement, decline in differentiation potential and MSC number with increased age, and low yield of MSCs upon processing.

3.1.4 Adipose-derived mesenchymal stem cells, ADSCs

ADSCs are mesenchymal stromal cells found in the perivascular space of adipose tissue. ADSCs have the advantage of abundance and easy access when compared with other stem cell types. ADSCs express common stem cell surface markers, genes, and differentiation potentials as MSCs[25]. ADSCs have demonstrated efficacy in experimental studies of urologic conditions [26]. Hung *et al* reported that isolated human ADSCs could be induced to differentiate into fibroblast, which was suitable to fabricate tissue-engineered fascia equivalents after implanted into a PLGA-based scaffold[27]. Recent study by Wu *et al* human ADSCs reseeded on ABP in vivo may further enhance the properties of ABP and promoted muscularis regeneration and neovascularization after implanted into a subcutaneous pocket, resulting in a promising treatment option for POP[28].

3.1.5 Endometrial mesenchymal stem cell, EMSCs

More recently, some studies have identified a unique source of human mesenchymal stem cells from endometrium that are highly clono-genic and proliferative, self-renew and differentiate into multiple mesodermal lineages and demonstrated its potential characteristics for neo-tissue regeneration[29]. One advantage of human eMSC is the relative ease with which they can be obtained by an endometrial biopsy as an office-based procedure without the use of anaesthesia, which is significantly less painful or invasive than bone marrow aspiration or liposuction[29]. A study by Edwards *et al* carried out a trial that human EMSCs seeded on PG+A scaffold and implanted subcutaneously into a pocket. Their results showed that the seeded eMSCs altered collagen growth and organization around the scaffold mesh filaments and affected physiologically relevant tensile properties of the scaffold-tissue complex[30].

3.1.6 Muscle fiber fragments, MFF

Minced skeletal muscle grafts have a remarkable capacity for muscle repair, which was discovered and described in detail decades ago and recently reintroduced as a potential tissue engineering therapy for volumetric loss of muscle tissue[31]. The muscle cells of the minced fibers die, but some of the satellite cells survive, are activated by the inflammatory process, and divide into proliferating myoblasts that ultimately fuse to form new muscle fibers[31]. Several studies have demonstrated autologous fresh muscle fiber fragments with their associated satellites cells may be used instead of cultured myoblasts[32]. The procedure is simple and inexpensive and MFF can be harvested and implanted during the same surgical procedure making the method clinically feasible and attractive. In addition, the MFF also contain other stem cells and extracellular components of importance for formation of new connective tissue, vessels, and nervous supply, which could be beneficial in native tissue POP repair. A study by Jango *et al* showed that fresh muscle fiber fragments seeded on MPEG-PLGA scaffolds prior to implantation subcutaneously on the abdomen of rates could form new striated muscle after 8 weeks. This resulted indicated such an approach is a potential method for treating POP[33].

3.2 Biological scaffold and its use for the treatment of POP

3.2.1 Naturally derived materials

Natural biomaterials are typically decellularized and may be autologous (harvested from the patient), allograft (harvested from cadaveric tissue) or xenograft (harvested from animal tissue)[34]. The biological scaffolds are obtained from tissues that have been treated to remove cellular components. It is thought that these scaffolds are more similar to the host scaffold and, present excellent biocompatibility and growth-promoting abilities, which make them interesting candidates for tissue engineering approaches[35]. The best studied natural decellularized materials are small intestinal submucosa (SIS), which have been used variously in experimental bladder reconstruction studies[36]. A study by Wu *et al* investigated the potential effect of using bovine pericardium matrix implant reseeded with hADSCs for the treatment of POP. Their results showed that the ABP scaffold could provides an optimal microenvironment for cells to attach and grow and the neovascularization of the whole construct formed after the substance was implanted subcutaneously for 12 weeks[28]. It is tempting to recall that fresh autografts of fascia lata or rectus fascia have been used successfully in reconstructive POP surgery or as suburethral slings for SUI treatment. Although originally conceived otherwise, these approaches in many ways mimic tissue engineering strategies. Fresh autologous fascia tissue provides three-dimensional structure, support, regenerative cells, and biocompatibility, as evidenced by the effects and safety of these treatments In a recent study, Mangera *et al*, tested various scaffolds and seeding with fibroblasts for tissue engineering for pelvic floor disorders and found that porcine small intestinal mucosa (SIS) and PLA scaffolds supported good cell attachment and had biomechanical properties closest to native tissue[17]. However, the disadvantages of all biological materials derived from animals or humans such as limited availability, high cost variable host tissue response, and concerns for disease transmission exist.

3.2.2 Synthetic polymers

Synthetic biomaterials are available in many varieties and are the current preferred material that can be manufactured under controlled circumstance for the treatment of POP[34]. During the procedure of the materials synthesized, the degradation time and the biomechanical properties of the material can be engineered to mimic normal biomechanics of the pelvic floor. Generally synthetic biomaterials may be characterized by poor size, filament type, local tissue durability and stiffness[37]. The main disadvantages of synthetic meshes are the complications of erosion (graft material in the urethra or bladder) and extrusion (graft material in the vaginal lumen). The rates of erosion and extrusion are variable and are related to a combination of host, operative and biomaterial factors. Host factors, such as impaired healing from micro-vascular disease, tobacco abuse, or early resumption of intercourse may promote extrusion.

3.3. Trophic factors

Cell-based tissue engineering therapy may benefit from the addition of additional bioactive molecules to the cell scaffold complex. The factors could enhance regenerative processes by initiating pathways for activation and recruitment of transplanted, resident, or circulating stem cells. For example, acellular tissue matrices such as SIS consist of abundant extracellular matrix, which could secrete a variety of growth factors, including basic fibroblast growth factor (BFGF) and transforming growth factor-β (TGF-β), as well as several glycosaminoglycans and other molecules of the extracellular matrix known to influence cell and tissue growth[38]. For example, Lin *et al* reported that SIS could significantly enhance the release of several angiogenic factors, including vascular endothecial growth factor (VEGF) and IL-8[39]. Preclinical and clinical studies show that estrogens also play a role in maintaining vaginal and pelvic floor supportive tissue by influencing fibroblast proliferation and collagen synthesis[40]. However, the importance of estrogen status in the development of POP is controversial. Takacs *et al*. showed that estrogen promoted growth of vaginal smooth muscle cells while Chakhtoura *et al* demonstrated that estrogen inhibited vaginal tropoelastin and TGF-β1 production[41, 42]. In addition, nerve growth factor enrichment of injectable PLGA microspheres and concomitant injection of ADSC improved urinary sphincter function in an SUI rat model, and this concept could be translated to treating POP, since PLGA may also be processed into a mesh (Vicryl)[43]. As new and safe procedures are emerging, gene transfer therapy may also be added to the tissue engineering approach, as demonstrated in orthopedic research by promoting the heal of osteochondral defects using plasmids for bone morphogenetic protein. In POP, imaging techniques

reveal that some patients have large muscular pelvic floor defects, and surgical techniques used currently do not repair these defects. In addition, accumulating evidence suggests that the metabolism of the vaginal tissue is abnormal in POP. Facilitated endogenous repair with local gene transfer could be useful in these patients as a causal treatment to correct the abnormalities.

4. Animal models

Animal models are particularly appropriate for studying the natural progression of pathologies and investigating novel treatment approaches. However, the development of applicable animal models for POP is challenging since humans, as the only strict bipeds, have particularly difficult childbirth delivery process and a unique pelvic orientation with regard to gravity. Several animal species, such as mice, rabbits, nonhuman primates (NHP), sheep, cows, pigs, dogs, domestic cats, deer, bongos, horses, buffalos, donkeys and cheetahs have been documented to spontaneously develop forms of POP. Although most of these species are not conducive for use in laboratory research, over the past few decades several of these species have been extensively studied and may serve as valuable animal models. Rodents, the most widely used animal models for investigation of the development of spontaneous POP in women, represent several anatomical differences with humans that must be considered when interpreting results from rodent studies. For example, the pelvic floor is oriented horizontally in rodents and they have a smaller fetal head-to-birth canal ration as compared to humans[44]. Rabbits have been reported to be appealing to study POP in a small mammal model. However, the anatomy of the rabbit vagina differs significantly from that of humans. For example, the vagina of the rabbit is extensive, consisting of both an internal and external portion. The former is more similar to the small intestine in gross and histological structure, and the latter contains a large portion of the anterior wall. Because of these differences in anatomy with human, the best use of rabbit models may be to study biocompatibility of surgical implants[45]. Sheep has been well documented to acquire spontaneous POP, which is the only domesticated animal that frequently suffers antepartum POP. The sheep as a model of prolapse presents several advantages such as similar normal anatomy, anatomical derangement in prolapse and similar risk factors. However, when using sheep, several logistic disadvantages must be considered, particularly the space required to board sheep[46]. So far, other animal models including cows, pigs, rhesus macaque, and squirrel monkey have been studied as a model of POP. However, it must be pointed that only rat model of POP was used to study the application of tissue engineering. Further studies were needed to evaluate the potential value of tissue engineering in other animal models.

5. Conclusion

POP constitutes a variety of common disorders affecting millions of women worldwide. Current treatments are limited and do not address the underlying pathophysiology of disorder or disease, nor do they repair damaged tissue to restore normal function. In contrast to current therapies, tissue engineering have emerged as an exciting treatment avenue targeting disease progression and potentially correcting pathophysiology. Although many challenges remain to be addressed before clinical implementation of this technology, preclinical animal show promise in the use of tissue engineering for POP.

Conflicts of interest

The authors report no conflict of interest.

Financial disclaimers

None.

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