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Review Article

Biomaterials for Hernia Repair in Animals; a Review

Abdul Mohsina[1], Paramasivam Tamilmahan[1*], Dayamon David Mathew[1], Vellachi Remya[1], Ninu Ajantha Ravindran[1], Naveen Kumar[1], Swapan Kumar Maiti[1], Kumaragurubaran Karthik[2], Manjunathachar Haranahalli Vasanthachar[3]

[1]Division of Veterinary Surgery, Indian Veterinary Research Institute, Izatnagar, India–243122; [2]Division of Veterinary Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, India–243122; [3]Division of Veterinary Parasitology, Indian Veterinary Research Institute, Izatnagar, India–243122
*Corresponding author: drtamilmahan.bison@gmail.com

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ABSTRACT

Hernia is a common surgical affection of animals and its treatment may vary from simple herniorrhaphy to hernioplasty depending on the size of the defects. Because of associated complications, synthetic materials are now being replaced by biological materials like acellular dermal grafts, acellular diaphragm, acellular aorta etc. Seeding of either embryonic or mesenchymal stem cells on grafts has found to ameliorate the healing time and cosmetic appearance. Skin fibroblasts possess the ability to inhibit the invitro expansion of T lymphocytes. Like mesenchymal stem cells (MSC), fibroblasts also secrete modulatory molecules like PGE2 and nitric oxide. Stem cell seeded bioengineered acellular grafts proved to be more effective than non seeded grafts as they reduce the immunogenicity of grafts and this seems to be a growing field in hernial treatment. This review article discusses decellularization, tissue engineering and use of acellular collagen matrices and fibroblast seeded scaffolds in hernioplasty.

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INTRODUCTION

Hernia is defined as the protrusion of the contents of a body cavity through a normal or abnormal opening in the wall of that cavity either to lie beneath the intact skin or to occupy another adjacent body cavity. In most of the abdominal hernias the parietal peritoneum covers the herniated structure and is called the hernial sac, which protrudes through the hernial ring (Malangoni and Rosen, 2007). Abdominal hernia is a term used to describe a hernia through any part of the abdominal wall other than a natural orifice. Small hernial ring with reducible hernial contents can be treated by conventional methods (Stock, 1954). Repair of extensive abdominal wall defects and voluminous hernias in animals poses great difficulties mainly due to large sized hernial rings and immense distortion of hernial margin. Recurrence in these cases occurs frequently and the deformity gets even worse because of inadequate tissue at the site to enable a satisfactory closure. These problems can be overcome by use of special methods like hernioplasty with implantation of biomaterials.

The surgical procedure for reconstruction of abdominal wall defects has gone through a series of changes. Previously small abdominal wall defects were reconstructed by simply apposing muscles later came the era of synthetic prosthetics and meshes which proved to be proficient in case of large hernial defects. The most frequent complication leading to implant failure and recurrence is infection of the surgical site (Ingle–Fehr et al., 1997). To avoid these complications, the prosthetic material should be inert, but it should also support fibroplasia (Johnson, 1969). Mesh repair proved to be better than suture repair resulting in lesser recurrence rate, abdominal pain and complications (Burger et al., 2004; Penttinen and Gronroos, 2008).

Meshes can be placed either intraperitoneally or extraperitoneally. In the latter it can be placed either as inlay or outlay. The prosthetic material can be applied simply by physical pressure between the layers of abdominal wall (Stoppa and Rives, 1984), by suturing with non–absorbable material (Lichtenstein *et al*, 1989), absorbable material (Gianlupi and Trindade, 2004), clips (Read, 2011) or fibrin glue (Agresta and Bedin, 2008; Negro et al., 2011). One advantage of inlay technique is minimal dissection of soft tissue, thereby reducing devascularised tissue and its drawback is high recurrence rate. Outlay technique is having demerit of extensive soft tissue dissection (Penttinen and Gronroos, 2008). First biomaterial for hernia repair was silver mesh described by Witzel (1900). Later other materials were introduced like nylon (Acquaviva and Bourret, 1944), perlon (Kneise, 1953), polyethylene (Usher, 1958), silk mesh (Handley, 1963), etc. Among synthetic materials polypropylene is a suitable material for abdominal wall defect repair, because of the inert nature it can be used even in presence of infection and contamination (Bellon et al., 1997; Vilar et al., 2009).

Because of the complications encountered in the use of synthetic materials in hernioplasty they are now being replaced by biological materials like fibrin hydrogel, alginate, chitosan, hyaluronic acid and collagen based acellular grafts (Pereira et al., 2013). These are having excellent biocompatibility, biodegradability and weak antigenicity make collagen one of the most useful biomaterial (Lee et al., 2001). The cells present in the cellular

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graft are responsible for immunological rejection of the grafts (Gulati and Cole, 1994). Immunological reaction prompted by local and systemic T helper cells are responsible for the production of anti–inflammatory cytokines and non–complement fixing antibodies in case of small intestinal submucosa (Allman et al., 2001). Cartmell and Dunn (2000) advocated that removal cells from the graft may reduce the antigenicity of the graft. Gulati and Cole (1994) observed less immunogenicity and better tolerance of acellular grafts in rats and rabbits. Zhang et al., (2002) also found acellular dermal matrix of xenogenic origin as an ideal material with features like adequate compatibility and low absorption properties.

Fibroblasts play an important role in regeneration of new tissues due to their growth accelerating property of tissue cells by secreting several growth factors and extra cellular matrix (ECM). Primary mouse embryonic fibroblast (p–MEFs) is an attractive cell culture model owing to its unique characteristics like lack of immunogenicity and ability to act as feeder cells. In comparison to other primary explant cultures they can be established easily and maintained and proliferated rapidly resulting in exponential increase in cells from a single embryo within several days (Garfield, 2010). Major Histocompatibility Complex (MHC) Class II antigens present on the transplanted cells are responsible for graft rejection. Fibroblasts are relatively immunologically inert as they lack these surface molecules. Seeding of fibroblasts on the biomaterials not only improved healing time but also the cosmetic appearance (Lamme et al., 2000; Remya, 2012).

Prosthesis in Hernia Repair

Synthetic and biological materials can be used as prosthesis for the repair of large abdominal wall defects. The different synthetic materials used are polyester fabric (Shoukry et al., 1997), nylon, Dacron and stainless steel (Kanade et al., 1988; George and Mohammad, 1993), cotton mesh (Kanade and Kumar,1990), mosquito net mesh (Stephenson and Kingsnorth, 2011), vicryl mesh (Buchsbaum et al., 1985), polypropylene mesh (Kassam et al., 2014), expanded polytetrafluro ethylene (PTFE) (Kennedy and Matyas, 1999; Gillion et al., 1999), mersilene mesh (Riply and Mc Carnon,1994), Carbon fibers and Carbon sheet (Gangwar, 2002), oxidized generated cellulose, polyethylene glycol and hylan G–F20 (Altinli et al., 2011). A synthetic non–absorbable material Polypropylene mesh (PP), is the most widely used material for abdominal wall replacement and reinforcement during hernia repair (Bastos et al., 2006).

Prosthetic materials can be absorbable or non–absorbable. Non–absorbable synthetic meshes like polypropylene and PTFE provide good mechanical strength, but can lead to complications like bowel adherence and obstruction, fistula development, wound infection and seroma/ hematoma formation (Aldridge and Simson, 2001; Losanoff et al., 2002; Korenkov et al., 2002; Besim et al., 2002). Synthetic absorbable materials like Dacron and Teflon were widely used. Synthetic materials are now being replaced by biological materials like acellular dermal grafts (Gangwar, 2002; Purohit, 2008), acellular diaphragm (Perme, 2007; Kaarthick, 2011), acellular aorta (Kumar et al., 2012) etc because of the complications that resulted from their use in hernioplasty. Absorbable biological graft materials have better potential to combat infection and are found to be effective and superior in contrast to synthetic non–absorbable materials (Daghighi et al., 2013).

As an alternative biological materials can be used in the repair of defect. But cellular grafts may cause immunological reactions due to the presence of histocompatable antigens. These complications can be prevented by use of decellularized matrices and these are the latest alternative in this series. Biological materials reported to be used are small intestinal submucosa and acellular dermis (Dalla–Vecchia et al.,1999; Avella et al., 2011), tensor fascia lata, rectus abdominis fascia, latissimus dorsi muscle (Brennman et al.,1995), human duramater (Takahashi et al.,1994), porcine dermal collagen (Zhang et al., 2003), diaphragm (Varshney et al., 1990), autologus full thickness skin and dermis (Bhattacharya and Bose, 1998), decellularized porcine small intestinal submucosa (Turner et al., 2011), human acellular dermis (Bellows et al., 2007), human acellular collagen matrix (Dufrane et al., 2008), acellular dermal graft and glutaraldehyde treated acellular dermal graft (Gangwar, 2002), porcine acellular dermal matrix and porcine small intestinal submucosa (Liu and Bhatia, 2002; Zhang et al., 2003), collagen binding basic fibroblast growth factor loaded collagen scaffolds (Shi et al., 2011), fibroblast seeded PLLA (poly lactic acid) 3D scaffolds (Pu et al., 2010), decellularized dermal scaffolds seeded with autologus bone marrow derived mesenchymal stem cells (Zhao et al., 2012). Bovine parietal peritoneum for repair of ventral hernia in rat model was successfully done by Bastos et al., (2006).

Advantages of biological materials include their ability to ward off infection due to release of antimicrobial peptides and non–complement fixing antibodies, and induction of a mild inflammatory response, host cell migration and angiogenesis. Their disadvantages are their high cost, rapid break down, host foreign body reaction and loss of graft material in the infected regions (Bellows et al., 2007). Preparation of acellular collagen rich tissue matrices is done by physical, chemical or enzymatic manipulation of different tissues like bone, skin, blood vessel wall, bladder submucosa, small intestinal submucosa etc. (Yoo et al., 1998; Chen et al., 1999). These acellular collagen rich tissue matrices have less immunogenicity and better tolerance than cellular grafts due to their reduced antigenicity (Gulati and Cole, 1994). Antigenic epitopes, damage associated molecular pattern (DAMP) and DNA molecules present in the biological matrices are removed by decellularization and chemical cross–linking process (Bianchi, 2007; Lotze, 2007). p–MEF or mesenchymal stem cells seeded biological scaffolds are widely used now a days. p–MEFs act as a feeder layer as it secretes the necessary factors required for development of other cell types and it is having high proliferative and regenerative capacity (Garfield, 2010). In the surgical treatment of skeletal muscle restoration, biological scaffolds represent a promising option.

Biomaterials

Any natural or synthetic material, comprising a part or whole of a living structure or biomedical device which carry out a natural function, augments it or replaces it is defined as a biomaterial. Biomaterials have emerged into a science, in fifty years time period. Biomaterial science deals with the study of biomaterials (Ratner et al., 2004). Biomaterial science comprises various branches of medicine, tissue engineering, material science biology and chemistry. An

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ideal biomaterial should be inert completely, it should have sufficient strength to hold during healing, should have good handling characteristics, should be easily sterilisable, should be non–toxic, non–carcinogenic, non–teratogenic and inexpensive (Rousch, 2003).

Extracellular matrix is obtained after eliminating cells present in a tissue or organ to produce a structure containing only structural and functional proteins which are released from the inhabitant cells of the organ or tissue from which they are developed (Gilbert et al., 2006; Badylak et al., 2009). It forms the framework for organic scaffolds. One of the prime ingredients of ECM is glycosaminoglycans (GAGs) and is responsible for the water retention and gel function of ECM. It binds growth factors and cytokines (Badylak, 2002).

Even though ECM is present in every tissues and organs, for therapeutic applications they should be gathered from limited sources. The extracellular matrices have been derived from variety of tissues like small intestinal submucosa (Kumar, 2010; Aachoui and Ghosh, 2011), urinary bladder (Zhu et al., 2010; Dewangan, 2010; Eberli., 2011; Haichao, 2013), tendons (Longo et al., 2010;), ligaments (Kew et al., 2011), tracheal matrix (Zhang et al., 2012), pericardium, diaphragm (Perme, 2007; Kaarthick, 2011), skin (Purohit, 2008), fish swim bladder (Kumar, 2010; Remya, 2012) for tissue engineering.

The functional requirements of dermal substitutes are, it should safeguard the wound from infection and fluid loss, provide a firm and biodegradable mould for the formation of new dermal tissue, should enhance migration of cells, should be easy to handle and withstand tear forces. (Van der Veen et al., 2010). Biodegradability of biological graft materials can be reduced by creating irreversible cross–links between the inhabitant molecules. (Badylak et al., 2009). Cross linking of collagen matrix can be done by reagents like glutaraldehyde (Yannas,1996), di–isocyanate (Oliver et al.,1982) or diphenylephosphorilazide (Petite *et al*.1994). The definition of tissue engineering according to Langer and Vacanti (1993) is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ". MacArthur and Oreffo (2005) defined tissue engineering as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use."

Tissue engineering uses combination of cells, engineering and materials methods, and suitable biochemical and physio–chemical factors for replacing or improving biological functions (Langer and Vacanti, 1993). It was once classified as a sub–field of biomaterials , but now as it has gained immense scope and importance it can be considered solely as a field that needs further classification. While The term tissue engineering is closely associated with applications that repair or replace either portions of or whole of tissues (*i.e*., bone, cartilage, blood vessels, bladder, skin, muscle etc.) such that they retain mechanical and structural properties for its proper functioning.

For developing engineered tissues both *in–vitro* and *in–vivo* approaches can be adopted. In the *in–vitro* approach organs are created in tissue culture flask or bioreactors for implantation and replacement of diseased or damaged tissue, and have received special attention from the lay press. In the *in–vivo* approach an acellular biomaterial is created with clues conductive for tissue cell recruitment into the biomaterial and inductive of cell differentiation to form the needed tissue. Bioactive and a biopolymer backbone are often present in many fabrication engineered for implantation. Bioactives stimulate tissue cells to migrate, proliferate and differentiate. The function of biopolymers is to mechanically support cell migration and proliferation. If prepared from natural ECM, additional biological stimuli to support cell and tissue function may be provided by these scaffolds and hydrogels (Lutolf and Hubbell, 2005). Different functions provided by engineered tissue are structural (bone, cartilage, and skin) or metabolic (liver, pancreas), or both (Chapekar, 2000).

Collagen is still the protein of choice for biomaterials preparation due to its superior biocompatibility and low immunogenicity and collagen–based biomaterials are the ones which are most opted for. Collagen from various tissue sources can be extracted and assembled by combining with other molecules. It is also used in the laboratory as a decellularized ECM in fundamental studies or in medical applications as tissue replacement material. Tissue engineering has progressed rapidly in recent years and has now evolved as an alternative to transplantation of tissue/organ (Chapekar, 2000). It has provided drastic advances in all fields of surgery like ophthalmology (Chirila,2010; Hashimoto et al., 2010; Pang et al., 2010; Trese et al., 2012), orthopedic surgery (Ivkovic et al., 2011; Mahapatra and Khan, 2011; Khan et al., 2012), dental surgery (Bohi et al., 1998; Balasundaram et al., 2012; Payne, 2014), soft tissue surgery (Black et al.,1998), cardiovascular surgery (Schoen, 2011; Kurobe et al., 2012; Lam and Wu, 2012; Moroni and Mirabella, 2014) and neurosurgery (Huang et al., 2009; Cullen et al., 2011; Aronson et al., 2012;).

Decellularization

A decellularization procedure usually initiates with disintegration of cell membrane using physical treatments or ionic solutions, followed by enzymatic treatment which removes cellular constituents of ECM. Later cellular detritus of tissue is dislodged. Along with these procedures mechanical stirring can be performed to enhance the potency (Gilbert et al., 2006). For solubilizing cytoplasmic ingredients of the cell and for eliminating nucleic acids RNA and DNA , treatment with acidic and alkaline solutions can be done. Porcine small intestinal submucosa can be made acellular by treating with 0.10–0.15% (w/v) peracetic acid (PAA). This treatment effectively eliminates cellular components from thin ECM construct along with disinfecting them by invading microbes and microbial enzyme oxidation. (Hodde and Hiles, 2002).

Minimizing the immune reaction of hosts and lowering the antigenicity of graft by cross linking are the two different approaches utilized to reduce rejection of these materials (Rosenberg et al., 1987). Yannas (1996) demonstrated that antigenicity of collagen was quantifiable but the real actual significance of such antigenicity was found to be negligible because of minimal difference between collagen of different species. Such a minimal antigenicity was found to be shown by remaining cellular components and ECM matrix protein in tissues treated

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with glutaraldehyde. (Coito and Kupiec–Weglinsky, 1996). The acellular grafts were less immunogenic having better tolerance by allogenic hosts and equally effective as isograft (Gulati and Cole, 1994; Nagao et al., 2011).

Depending on the organs and tissues of interest the method of decellularisation alter extensively and different factors like tissue origin, distinct physical, chemical and enzymatic procedures determine the effectiveness of the procedure. The biochemical conformation, fine structure of tissue and instinctive nature of the resultant ECM scaffold will be affected by these treatment procedures which alter host response to the material. The objective of decellularisation procedure is to efficaciously eliminate all cellular and nuclear components along with diminishing detrimental impacts of conformation, biological functions and instinctive stability of the residual ECM (Gilbert et al., 2006).

The decellularized tissues retained their natural mechanical characters and by blood vessel proliferation and host cell migration the prosthesis was reconstructed (Schmidt and Baier, 2000). Class I and II histocompatibility antigens and glycoproteins present in extracellular matrices will be identified by immune system and have the ability to evoke rejection reactions. For preventing rejection reactions these components should be removed, but their entire removal is strenuous to execute and substantiate. (Malone et al., 1984; Wong and Griffiths, 2014).

Physical methods of decellularisation include snap freezing (Wang et al., 2012; Sheridan et al., 2013), mechanical agitation and sonication, applying direct pressure (Freytas et al., 2004). The chemical methods of decellularisation include use of acid and alkaline agents, Non–ionic detergents like triton X–100, ionic detergents like sodium dodecyl sulfate, triton X–200, zwitter ionic detergents like 3–[(3–cholamidopropyl)dimethylammonio]–1–propanesulfonate (CHAPS), sulfobetaine–10 and –16 (SB10, SB–16), Tri(*n*–butyl) phosphate, hypotonic and hypertonic solutions. The enzymatic method of decellularisation includes use of trypsin, endonucleases and exonucleases (Gilbert et al., 2006).

Solubilization of both cytoplasmic and nuclear cellular membranes can be efficiently done by ionic detergents and they denature proteins by deranging synergy between proteins (Seddon et al., 2004). Even though Sodium deoxycholate productively eliminate cellular residues, it induces substantial disturbance to normal tissue framework in contrast to sodium dodecyl sulphate (Gilbert et al., 2006). For disintegrating cells within tissues and organs osmotic shock of hypotonic solutions like deionized water can be utilized. (Woods and Gratzer, 2005).

Fibroblasts

The fibroblasts are cells usually existing in connective tissues and they constantly release predecessors of extracellular matrix like collagens, glycosaminoglycans, reticular and elastic fibers and glycoproteins in mammalian tissues. Skin fibroblasts have the ability to hinder the *in vitro* multiplication of T lymphocytes. Like mesenchymal stem cells, fibroblasts produce modulatory molecules PGE2 and nitric oxide. (Bouffi et al., 2011).

A thin three dimensional sheet of ECM material can be created *in–vitro* by inducing fibroblasts. (Ishikawa et al., 1997; Sakai et al., 2013). Fibroblasts remodel the collagen matrix from dendritic to stellate/bipolar, and punctuate cell matrix interactions are matured to form focal adhesion organization. Divya and Nandakumar (2006) found that collagen being chemotactic for fibroblasts, augments sequestration of fibroblasts through the fibrillar arrangement of scaffold and it enhances bonding of fibers and clot by prompting platelet degranulation *in vivo*. Fibroblasts grown on DMEM media were found to be spindle shaped, elongated and bipolar in nature and were having extensive projections showing contacts with neighbouring cells. Dubay et al., (2004) reported that by the lodgment of fibroblast growth factor liberating polyglactone polymer rod into the fascial wound of rat primary incisional hernias were lowered from 60 to 30% and recurrent incisional hernias from 80 to 23%. . In bFGF treated fascia type 1 collagen staining was found to be notably enhanced. Because of absence of HLA Class II antigens stem cells are having low immunogenicity and high immunosuppressive properties (Pereira et al., 2013).Stem cell seeded bioengineered acellular grafts proved to be more effective as they reduce the immunogenicity of grafts and this seems to be a growing field in hernial treatment.

CONCLUSION

Collagen based grafts are widely used degradable biological material for hernioplasty. They can be prepared from a variety of biological materials like skin, diaphragm, aorta, tensor fascia lata, latissimus dorsi muscle etc. Acellular collagen rich tissue matrices are prepared by physical, chemical or enzymatic manipulation of these tissues. Advantages of biological materials include their ability to combat infection, evoke a minimal inflammatory reaction, and enhance proliferation of blood vessels and migration of host cells. Stem cell seeded bioengineered acellular grafts proved to be more effective as they reduce the immunogenicity of grafts and this seems to be a growing field in hernial treatment. Biomaterials have been a gift to the field of surgery involving hernias as they can provide the support and in case of extensive muscular damages.

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