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Pericardial Processing: Challenges, Outcomes and Future Prospects

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1. Introduction

The pericardium is a biological tissue widely used as a biomaterial for tissue engineering applications, including the construction of a variety of bioprostheses such as vascular grafts, patches for abdominal or vaginal wall reparation and, more frequently, heart valves.

However, despite significant advances, some drawbacks have been found in these bioprostheses such as biological matrix deterioration and tissue degeneration associated with calcifications, even though xenopericardium or glutaraldehyde-treated autologous pericardium were used.

In non-autologous pericardial processing, the pericardium must be decellularized in order to remove cellular antigens and procalcific remnants while preserving extracellular matrix integrity. A large variety of decellularization protocols exist, such as chemical, physical or enzymatic methods. Additional cross-linking processing must be carried out to render the tissue non-antigenic and mechanically strong.

So far, almost all bioprosthetic materials made of pericardium, and used in clinical practice, are glutaraldehyde-treated bovine or porcine xenopericardium. However, long-term reports are raising issues concerning their durability, especially highlighting the high risk of calcification. Regarding heart valves, calcification currently represents the major drawback leading to potential failure of the bioprosthesis.

The aim of this review is to present current issues, challenges, outcomes and future prospects of pericardial processing, including decellularization and cross-linking steps. Understanding current issues and improving pericardial processing will allow refining bioprosthesis conception and patients' safety.

2. Characteristics of the pericardium

2.1 Localization and composition

The pericardium is a connective tissue sac surrounding the heart. It is composed by two layers: a deeper layer closely adherent to the heart, the visceral serous pericardium, or

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epicardium, and an upper layer: the parietal pericardium. The two layers are separated by the pericardial cavity. The parietal pericardium can be excised and easily tested without causing major complications such as contracture or ischemia (Fomovsky et al., 2010).
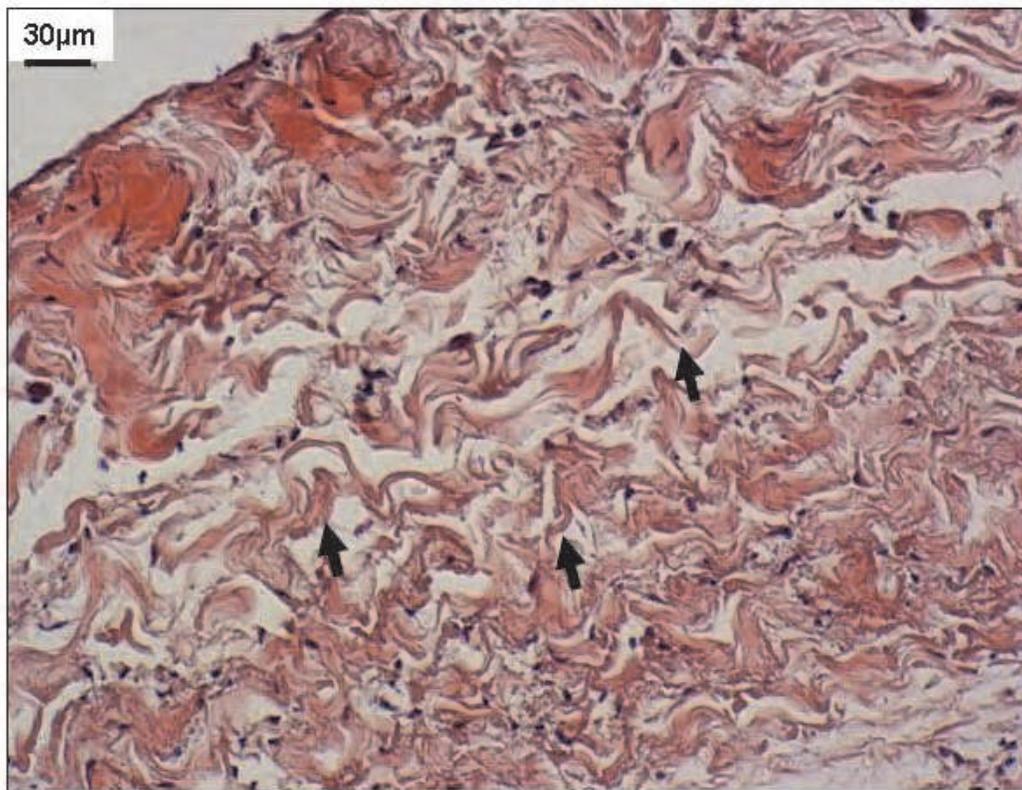

Fig. 1. Histology of ovine pericardium showing the collagen organization (arrows). Hematoxylin-Eosin staining.

The pericardium is composed of a simple squamous epithelium and connective tissue. It is a collagen-rich biological tissue containing mostly type I collagen, as well as glycoproteins and glycosaminoglycans (GAGs) in addition to its constitutive cells (Figure 1). Collagen is structured into different levels of organization ranging from fibrils to laminates, fibers and fiber bundles (Allen & Didio, 1984; Lee & Boughner, 1981). This organization determines the mechanical properties of the pericardial tissue (Sacks, 2003; Liao et al., 2005; Wiegner & Bing, 1981; Lee & Boughner, 1985) and provides an anisotropic and non-linear mechanical behaviour (Zioupos & Barbenel, 1994). Interestingly, depending on the location on the pericardium, the thickness and mechanical properties vary (Hiester & Sacks 1998a, 1998b). Thus, the location of the sample that will be harvested should be carefully selected when designing a tissue engineering protocol.

2.2 Sources of pericardium

Currently marketed heart valve bioprostheses are prepared from bovine or porcine pericardium (Vesely, 2005). Other pericardial tissues from different species have been assessed or are currently used in clinical practice such as equine (DeCarbo et al., 2010; Yamamoto et al., 2009; Sato et al.; 2008.), canine (Lee & Boughner ; 1981; Wiegner & Bing,

1981; 1985), or, even more unusually, ostrich (Maestro et al., 2006) or kangaroo pericardium (Neethling et al., 2000; 2002). However, those exogenous grafts raise several issues, and especially the immune response against the bioprosthesis as well as the viral status of the graft.

Human autologous pericardium is thus an interesting option, presenting several advantages over allografts since it is free of donor-derived pathogens and does not induce any immune response (Mirsadraee et al., 2007), is easily available, easily handled and of low cost. Ultimately, these characteristics allow for shorter and less aggressive pericardial processing before implantation of the bioprosthesis. However, because of intermittent reports of its tendency to retract or become aneurysmal, the general opinion has been negative (Edwards et al., 1969, Bahnson et al., 1970). For cusp tissue replacement or valve tissue replacement, stabilization of pericardium is performed with a solution of 0,2% to 0,6% glutaraldehyde in order to prevent secondary shrinkage (Duran et al., 1998; Al-Halees et al., 1998, 2005; Goetz et al., 2002).

3. Processing of pericardium

As allografts have been the main source for pericardial bioprostheses currently in use, significant processing steps have to be performed prior to clinical use. In particular, as xenogeneic cellular antigens induce an immune response or an immune-mediated rejection of the tissue, decellularization protocols are widely used to reduce the host tissue response (Gilbert et al., 2006.). Once decellularized, the free-cell pericardial tissue is composed of extracellular matrix proteins which are generally conserved among species, and thus can be easily used as a scaffold for the host cell attachment, migration and proliferation (Schmidt & Baier, 2000). This scaffold considerably accelerates tissue regeneration. Overall, tissue decellularization aims at reducing tissue antigenicity and host response while preserving the mechanical integrity, biological activity and composition of the ECM (Simon et al., 2006; Gilbert et al., 2006).

3.1 Extracellular matrix decellularization methods

Most decellularization protocols include a combination of various methods, such as physical, enzymatic or chemical treatments (Gilbert et al., 2006; Crapo et al., 2011). Physical methods can either rely on snap freezing (Jackson et al., 1988; Roberts et al., 1991), mechanical force (Freytes et al., 2004) or mechanical agitation (Schenke-Layland et al., 2003), whereas enzymatic protocols employ nucleases, calcium chelating agents or protease digestion (Teebken et al., 2000; Bader et al., 1998; McFetridge et al., 2004; Gamba et al., 2002). Regarding physical decellularization processes, sonication, based on the use of ultrasounds to disrupt the cell membrane, has been investigated. Such treatment considerably affects the pericardial architecture and full decellularization cannot be achieved. Thus sonication has to be carried out simultaneously with chemical treatments in order to fully decellularize the pericardial tissue and remove cellular debris. However, this combination leads to alterations of the extracellular matrix (ECM) architecture.

For the enzymatic procedure, the main enzyme employed is trypsin, cleaving peptide bonds on the C-side of arginine and lysine and thus allowing separation of the cells from the ECM.

Chemical protocols involve use of alkaline and acid treatments (Freytes et al., 2004), ionic detergents, sodium dodecyl sulfate (SDS), sodium deoxycholate and Triton X-200 (Rieder et

al. 2004; Hudson et al., 2004), non-ionic detergents, such as Triton X-100 (Grauss et al., 2003), zwitterionic detergents (Dahl et al., 2003), tri(n-butyl)phosphate (Woods & Gratzer, 2005) as well as hypertonic or hypotonic solutions (Goissis et al., 2000; Woods & Gratzer, 2005; Vyavahareet al., 1997; Dahl et al., 2003). These modalities will either mediate lysis of the cells or solubilization of the cellular components.

Overall, standard decellularization protocols for allografts consist of a multimodal process starting with the lysis of the cell membrane using either ionic solutions or physical treatments. This initial step is then followed by enzymatic treatments to separate any cellular components from the ECM. Subsequently, detergents are used to solubilize the nuclear and cytoplasmic cellular components. At the end of the procedure, all residual cell debris is removed from the remaining ECM. A washing step must also be carried out following the decellularization protocol to remove residual chemicals, thus avoiding any host tissue response (Gilbert et al., 2006). The efficiency of the decellularization protocol and the preservation of the ECM have to be assessed using histological tools.

Concerning pericardial decellularization, several protocols, which have provided interesting results, can be found in the literature. (Courtman et al., 2004; Liang et al., 2004; Wei et al., 2005; Chang et al., 2005; Mendoza-Novelo et al., 2010, Ariganello et al., 2011). Courtman *et al.* proposed the use of a non-ionic detergent, Triton X-100 and an enzymatic extraction process. After this treatment, the acellular matrix was shown to be composed of collagen, elastin and glycosaminoglycans (GAG). Microscopy revealed that all cellular components were removed and that matrix ultrastructure was intact. More recently, Mendoza-Novelo *et al.* compared the surfactant tridecyl alcohol ethoxylate (ATE) and the reversible alkaline swelling (RAS) treatments to Triton X-100 (Mendoza-Novelo et al., 2010). Histological results indicated a significant reduction of cellular antigens with these three decellularization processes. Nevertheless, the native GAG content varied significantly. It decreased from 88.6 ± 0.2% to 62.7 ± 1.1% and 61.6 ± 0.6% for RAS treatment, ATE and Triton X-100 respectively.

On human pericardial tissue, Mirsadraee *et al.* used a protocol employing hypotonic buffer, SDS, protease inhibitors and nuclease solution. Following decellularization, the tissue is decontaminated using a peracetic acid solution (Mirsadraee et al., 2006; 2007). With this process, glycosaminoglycans and structural proteins, such as collagen, remained intact.

Finally, when dealing with autologous pericardium grafting, full decellularization might not be necessary and thus, simpler protocols can be used. For instance, surgeons commonly prepare autologous pericardium for heart valve replacement by mechanical friction. This allows removing sub-pericardial fat before implantation while better preserving the pericardial architecture stability. This mechanical treatment mainly removes superficial cells, thus allowing 50% of viable pericardial cells to remain in the graft (personal data). The preservation of the pericardial architecture as well as part of the pericardial cells, should maintain a better integrity of the graft, while allowing re-cellularization of the superficial layers.

3.2 Effects of decellularization

Depending on the protocol, decellularization may have an impact on the structural and mechanical properties of the treated tissue (Gilbert et al., 2006). According to Zhou *et al.*, decellularization protocols differ significantly in terms of alteration of ECM histoarchitecture (Zhou et al., 2010). For instance, decellularization protocols have a strong impact on the amount of GAGs remaining in a tissue (Badylak et al., 2009; Mendoza-Novelo et al., 2010). Removing GAGs from a tissue leads to adverse effects on pericardial

viscoelastic properties. This can be easily understood since water retention is an important function of GAGs in tissues (Lovekamp et al., 2006). Moreover, GAG content plays a key biological role in cellular signaling and communication. Thus, decreasing GAG content leads to an impaired tissue response and repair. Therefore, the decellularization protocol has to be carefully chosen depending on the tissue type as well as the targeted application. Ideally, the process should remove all cellular antigens without compromising the structure and mechanical properties of the tissue.

Liao *et al.* (Liao et al., 2008) investigated the effect of three decellularization protocols on the mechanical and structural properties on porcine aortic valve leaflets. These protocols were based on the use of SDS, Trypsin and Triton X-100. They showed that decellularization resulted in collagen network disruption, and that the ECM pore size varied as a function of the protocol used. For example, leaflets treated with SDS displayed a dense ECM network and small pore sizes, characteristics that may have an impact on the recolonization of interstitial cells.

It has been demonstrated that decellularization of bovine pericardium with SDS causes irreversible denaturation, swelling and a decrease in tensile strength compared to native tissue (Courtman et al. 1994; García-Paéz et al., 2000; Mendoza-Novelo et al., 2009). Because of these deleterious effects on pericardial tissue, non-ionic detergents are preferred for decellularization processes (Mendoza-Novelo et al., 2010). Nevertheless, some issues may be encountered with the use of non-ionic detergents. Indeed, toxic effects (Argese et al., 1994) and estrogenic effects (Soto et al., 1991; Jobling et al., 1993) have been reported after the use of non-ionic detergents such as alkylphenol ethoxylates.

Decellularization mediates alterations of the structural and mechanical properties of the tissue, but this impact varies depending on the protocol used. For instance, Mirsadraee *et al.* (Mirsadraee et al., 2006) did not observe any significant changes using an SDS-based decellularization protocol in the ultimate tensile strength compared to native tissue on human pericardial tissue. They also observed an increased extensibility of the tissue when cut parallel to collagen bundles.

Tissue decellularization reduces the cellular and humoral immune response targeted against the bioprosthesis (Meyer et al., 2005). However, removing cells does not ensure adequate removal of xenoantigens, nor mitigation of the immune response (Goncalves et al., 2005; Kasimir et al., 2006; Simon et al., 2003; Vesely et al., 1995). For this reason, decellularization protocols have turned to antigen removal protocols (Ueda et al., 2006; Kasimir et al., 2005). The presence of cell membrane antigens, such as oligosaccharide beta-Gal has been reported to lead to an immune response that can be prevented by effective decellularization (Badylak et al., 2008). Interestingly, Griffiths *et al.* (Griffiths et al., 2008) used an immunoproteomic approach to study the ability of bovine pericardium to generate a humoral immune response. They identified thirty one putative protein antigens. Some of them, such as albumin, hemoglobin chain A and beta hemoglobin have been identified as xenoantigens. Recently, Ariganello *et al.* provided evidence that decellularized bovine pericardium induced less differentiation of the monocytes to macrophages compared to polydimethylsiloxane or polystyrene surfaces (Ariganello et al., 2010; 2011). Nevertheless, the effects of the host immune response to acellular pericardium remain to be fully characterized. Understanding this phenomenon is necessary to develop new pericardium preparations and thus improve biological scaffold integration and clinical safety (Badylak & Gilbert, 2008).

Overall, no optimal decellularization treatment has been identified so far, but depending on the target tissue as well as the implantation site, the protocol can be adapted to provide the

best decellularization efficiency / functional characteristics ratio. Moreover, some additional treatment can be performed following the decellularization step in order to improve the mechanical and biological features of the graft.

4. Pericardial extracellular matrix treatment

The decellularization process will lead to important alterations of the biomaterial. Its mechanical strength will be diminished and after implantation it will undergo rapid resorption. Hence, approximately 60% of the mass of the ECM is degraded and resorbed between one and three months after *in vivo* grafting (Badylak & Gilbert, 2008). It has also been noted that acellular pericardial tissue, mostly made of type I collagen, is highly thrombogenic (Keuren et al., 2004). Finally, preventing calcification of the graft is also a priority to ensure the long-term benefit of the implantation.

To optimize the features of the bioprosthesis before its clinical grafting, several treatments have been developed and are summarized in Table 1.

	Reagents	References
Cross-linking treatment	Acyl azide	(Petite et al., 1990)
	Carbodiimides	(Sung et al., 2003)
	Cyanimide	(Pereira et al., 1990)
	Dye-mediated photooxidation	(Moore et al., 1994)
	Epoxy compound	(Sung et al., 1997)
	Formaldehyde	(Nimni et al, 1988)
	Genepin	(Sung et al., 1999, 2003; Wei et al., 2005)
	Glutaraldehyde	(Huang-Lee et al, 1990; Jayakrishnan et al., 1996; Thubrikar et al., 1983)
	Glutaraldehyde acetals	(Yoshioka et al., 2008)
	Penta-golloyl glucose	(Tedder et al., 2008)
	Phytate	(Grases et al., 2006, 2008)
	Proanthocyanidin	(Han et al., 2003)
	Reuterin	(Chen et al., 2002)
	Tannic acid	(Cwalina et al., 2005; Jastrzebska et al., 2006; Wang et al., 2008)
Coating treatment	Chitosan	(Nogueira et al., 2010)
	RGD polypeptides	(Dong et al., 2009)
	Silk fibroin	(Nogueira et al., 2010)
	Heparin sodium	(Lee et al., 2000)
	Titanium	(Guldner et al., 2009)
Post-fixative treatment	Amino acids	(Jorge-Herrero et al., 1996; Moritz et al., 1991)
	Glycine	(Lee et al., 2010)
	Heparin	(Lee et al., 2000, 2001)
	Hyaluronic acid	(Ohri et al., 2004)
	L-arginine	(Jee et al., 2003)
	L-glutamic	(Grimm et al., 1991; Leukauf et al., 1993)
	Lyophilization	(Santibáñez-Salgado et al., 2010)
	Sulphonated poly(ethylene oxide)	(Lee et al., 2001)

Table 1. Pericardial processing.

4.1 Cross-linking treatment of pericardial tissue

Cross-linking processing must be carried out to render the tissue non-antigenic, mechanically strong and to minimize xenogeneic tissue degradation (Eliezer et al., 2005; Love, 1997). Nevertheless, degradation should not only be considered as a negative phenomenon, as low molecular weight peptides formed during ECM degradation may have a chemo-attractant potential for several cell types (Badylak & Gilbert, 2008). It is thus the degradation rate of the scaffold that should be primarily considered and evaluated. Depending on the application and cells involved, the degradation rate has to be investigated to ensure proper host cell recruitment and tissue remodelling. The pathways of the immune response involved in this process remain to be fully described (Badylak & Gilbert, 2008).

Introducing cross-links between the polypeptide chains of the ECM has been shown to reduce immunogenicity of the pericardium (Mirsadrae et al., 2007) as well as its biodegradability (Taylor et al., 2006) by increasing its resistance to enzymatic degradation.

Until now, glutaraldehyde (GA)-fixed bovine pericardium has been preferred as a substitute to autologous human pericardium. GA was first introduced by Carpentier *et al.* (Carpentier et al., 1969) as a cross-linking reagent to chemically modify the collagen and render the tissue immunologically acceptable in the human host. Fixation was shown to increase stability and strength of the pericardium (Jayakrishnan & Jameela, 1996). GA remains the gold standard as a cross-linking reagent despite its well-known drawbacks. Indeed, GA has been reported to accelerate the calcification process, which considerably limits its application. Calcification is thus the main cause of long-term failure of GA-fixed pericardial valves (Gallo et al., 1985; Grabenwoger et al., 1996). Furthermore, a GA-treated pericardium has a poor ability to regenerate *in vivo* due to the cross-linking of the tissue. Moreover GA residues display cytotoxic effects preventing host cell attachment, migration and proliferation (Huang-Lee et al., 1990).

It is now accepted that GA cross-linking increases tissue stiffness (Thubrikar et al., 1983) with the possibility of tissue buckling (Vesely et al., 1988). Standard use of GA cross-linking leads to a high risk of calcific degeneration as well as tissue fatigue (Grabenwoger et al., 1992). This is mostly due to inflammatory and cytotoxicity changes (Huang Lee et al., 1990), and continuous wear and tear leading to collagen fiber fragmentation.

Besides glutaraldehyde, several cross-linking compounds have been reported in the literature such as genipin (Wei et al., 2005) or epoxy compound (Sung et al., 1997). These alternative methods are used to bridge hydroxylysine residues of different polypeptide chains or amino groups of lysine by oligomeric or monomeric crosslinks (Sung et al., 2003). Because of the adverse effects of cross-linking with glutaraldehyde or other aldehyde treatments such as formaldehyde (Nimni et al., 1988) or dialdehyde starch (Rosenberg, 1978), numerous non-aldehyde treatments have been proposed, such as carbodiimides (Sung et al., 2003), glycerol (Ferrans et al., 1991), glycidal ethers (Thyagarajanet al., 1992) including poly(glycidylether) (Noishiki et al., 1986), acyl azide (Petite et al., 1990), cyanimide (Pereira et al., 1990), genipin (Wei et al., 2005), or dye-mediated photo-oxidation, phytate (Grases et al., 2008).

Genipin, obtained from the fruits of *Gardenia jasminoides* ELLIS (Fujikawa et al., 1987; Tsai et al., 1994), exhibited better results than glutaraldehyde regarding its cytotoxicity (Sung et al., 1999), inflammatory response, ability to prevent calcification and tissue-induced mechanical properties (Wei et al., 2005). Epoxy compound, initially proposed by Noishiki *et al.* (Noishiki

et al., 1989), was shown to be less cytotoxic, superior in pliability and to better inhibit calcification than glutaraldehyde (Sung et al., 1997).

Carbodiimides generate amide-type crosslinks via direct cross-linking of the polypeptide chains. Use of carbodiimide cross-linking leads to the activation of the carboxylic acid groups of glutamic or aspartic acid residues to obtain O-acylisourea groups. Hydroxyline residues or free amino groups of lysine generate a nucleophilic attack which allows cross-link formation (Timkovich, 1977). It was noted that adding N-hydroxysuccinimides to carbodiimides considerably increases cross-link number (Olde Damink et al., 1996). In addition, the use of carbodiimides displayed increased stability towards enzymatic degradation on collagen-based tissue such as pericardium (Sung et al., 2003).

Glutaraldehyde acetal cross-linking reagent has been developed with glutaraldehyde in acid ethanolic solution (Yoshioka & Goissis, 2008), protecting free aldehydic reactive groups and minimizing the polymeric formation of glutaraldehyde. This reduces superficial effects with glutaraldehyde cross-linking on pericardial tissue.

Crosslinking of the pericardial tissue with a dye-mediated photo-oxydation process provides chemical, enzymatic and *in vivo* stability as well as biomechanical integrity of the treated tissue (Moore et al., 1994). Penta-golloyl glucose, a collagen-binding polyphenol, stabilizes collagen, preventing its degradation, and allows progressive host cell infiltration as well as ECM remodeling. An *in vivo* study has shown that porcine pericardium does not calcify with such treatment at 6 weeks when implanted subdermally in rats (Tedder et al., 2008). Reuterin, an antimicotic and antibacterial compound obtained from *Lactobacillus reuteri* (Axelsson et al., 1989), has been studied as a crosslinking reagent (Chen et al., 2002). It is a three-carbon aldehyde reacting, as formaldehyde, with free amino groups. Reuterin cross-linked pericardium exhibits comparable results to glutaraldehyde in terms of resistance against enzymatic degradation, denaturation temperature and free amino group content, while decreasing cytotoxic effects (Chen et al., 2002). Tannic acid has been studied on pericardial tissue and was shown to crosslink proteins by creating multiple hydrogen bonds due to its hydroxyl groups (Cwalina et al., 2005; Jastrzebska et al., 2006). It exerts an anti-inflammatory effect, especially on macrophages, as well as an anti-calcification effect on glutaraldehyde-fixed bovine pericardium (Wang et al., 2008). Proanthocyanidin, a natural crosslinking reagent with polyphenolic structures, has the potential to create a stable hydrogen-bonded structure and to increase collagen synthesis, generating nonbiodegradable collagen matrices (Han et al., 2003). Proanthocyanidin-treated pericardial tissues are non-cytotoxic and resist against enzyme digestion, and have been shown to be compatible with cell attachment and proliferation. Phytate has been suggested as an anti-calcification reagent (Grases et al., 2006, 2008) and has achieved promising results, to be validated by further studies. Other amide-type crosslinks, based on the activation of carboxyl groups, have been studied, such as diphenylphosphorylazide or ethyldimethylaminopropyl carbodiimide. It appears, according to Jorge-Herrero *et al.*, that these two chemical treatments are not a good alternative compared to glutaraldehyde. Indeed, pericardial tissues treated with those reagents are less resistant to calcifications and proteolytic attacks (Jorge-Herrero et al., 1999).

Numerous alternative treatments to glutaraldehyde cross-linking have been developed and investigated over the years. However, most of them were mainly evaluated *in vitro* and compared only to glutaraldehyde. A comprehensive comparative study of the different reagents remains to be conducted in terms of benefits regarding the tissue properties as well as their potential toxicity or deleterious effects.

4.2 Coating of the pericardium

Another possible post-decellularization treatment resides in the coating of the bioprosthesis. This procedure should allow improvement of graft integration at the site of implantation as well as decreasing degradation of the pericardial tissue.

Coating bovine pericardium with biopolymeric films, either chitosan or silk fibroin, has been investigated by Nogueira *et al.* (Nogueira et al., 2010). These methods are interesting approaches and both treatments appear to be non-cytotoxic. Nevertheless, chitosan does not allow endothelialisation and silk fibroin-coated bovine pericardium calcifies *in vivo*. Further investigation has to be performed to tackle these major concerns.

In their study, Dong *et al.* suggested treating bovine pericardium with acetic acid coupled with RGD polypeptides (Dong et al., 2009). Acetic acid increases pericardial scaffold pore size and porosity while RGD peptides is meant to improve cell adhesion and growth. Hence, RGD polypeptides have been identified in fibronectin (Pierschbacher & Ruoslahti, 1984), collagen, vitronectin and membrane proteins (Ruoslahti & Pierschbacher, 1987). These sequences have an impact on integrins, which display cell adhesion receptor roles controlling cell signaling pathways.

4.3 Pericardium anti-calcification treatments

The mechanism of calcification on glutaraldehyde-treated pericardium is not well understood because of its complexity. Nevertheless, there is evidence that pericardial tissue residual antigens, free aldhehyde groups of glutaraldehyde and phospholipids are involved in this mechanism.

Thus, circulating antibodies can contribute to pericardial calcification due to a possible immune response. Free aldehyde groups of glutaraldehyde can attract host plasma calcium, increasing tissue calcification. Phospholipids may bind calcium and play an important role in the calcium phosphate crystal formation. Several strategies have been investigated to tackle these major issues.

Suppression of residual antigenicity has been proposed to prevent calcification and it has been shown to be effective. This was performed by fixation treatments using a broad range of high concentrations of glutaraldehyde (Trantina-Yates et al., 2003; Zilla et al., 2000). To remove free aldehyde groups, a large number of amino acids or amino compounds were studied. Post-fixation treatments with amino acids displayed an improved spontaneous endothelialisation *in vivo* of glutaraldehyde-fixed bovine pericardium (Moritz et al., 1991; Jorge-Herrero et al., 1996). The use of L-glutamic acid did reduce residual and unbound aldehyde groups, on glutaraldehyde-fixed bovine pericardium and significantly decreased the risk of calcification (Grimm et al., 1991; Leukauf et al., 1993). Post-treatment with L-arginine also resulted in decreased calcium deposition (Jee et al., 2003). Recently, Lee *et al.* proposed a post-fixation treatment with glycine (Lee et al., 2010). Early results are promising but require further investigation on larger studies.

Alcohol solutions, including ethanol, have been investigated as a treatment to remove tissue phospholipids, thus preventing calcification (Pathak et al., 2004; Vyavahare et al., 1998). Besides, other techniques have been proposed to minimize the side effects of glutaraldehyde residues on GA-treated pericardium. Lyophilization has been shown to decrease aldehyde residues, decreasing the risk of calcification and cytotoxicity (Santibáñez-Salgado et al., 2010).

Moreover, treatments with heparin or sulphonated poly(ethylene oxide) following glutaraldehyde pre-treatment have been proposed (Lee et al., 2000, 2001). Both methods block side effects of GA residues and thus prevent calcification of the pericardium. Finally, a

modified adipic dihydrazide hyaluronic acid has been proposed to be grafted on to glutaraldehyde-treated bovine pericardium (Ohri et al., 2004). Calcifications decreased considerably with this post-treatment compared to the control group at two weeks following a subcutaneous implantation in mice.

5. Applications of the pericardium as a biomaterial

So far, the pericardium has been mostly used for cardio-vascular applications, i.e. vascular grafts (Schmidt & Baier, 2000; Chvapil et al., 1970; Matsagas et al., 2006; Menasche et al.,

Pericardium source	Surgical fields	Product	Company
Bovine or porcine	Soft tissue repair Hernia repair Abdominal & thoracic wall defects	-Peripatch® Implantable surgical tissue -TutoMesh®	Neovasc, Maverick Biosciences PTY Limited, Tutogen medical GmbH, RTI Biologics, Med&Care, Biovascular Inc, Novomedics
	Strip reinforcement	-Veritas Peristrips® Dry	Synovis Life Technology
	Orbital repair	-Tutopatch® -Ocugard®	Tutogen medical GmbH, RTI Biologics, Med&Care, Biovascular Inc, Novomedics
	Dural repair	-Lyolem ®r All BP	National tissue Bank Malaysia
	Perivascular Patch	-Peripatch® biologic vascular patch	Neovac
	Cardiac reconstruction and repair	-Peripatch® Implantable Surgical Tissue	Neovasc, Maverick Biosciences PTY Limited
	Heart valve replacement	-PercevalS® aortic valve -Mitroflow® pericardial aortic valve -Freedom solo® -Carpentier-Edwards PERIMOUNT® Magna EaseAortic Heart Valve -Carpentier-Edwards PERIMOUNT® Magna Mitral Ease Heart Valve -Carpentier-Edwards PERIMOUNT® Theon Aortic Heart Valve -Carpentier-Edwards PERIMOUNT® Theon Mitral Replacement System	Sorin group " " Edwards Life Sciences " " "
Equine	Tendon repair	-OrthADAPT®	Synovis Life Technologies Inc
Human	Valvuloplasty Heart valve	-Xeno or (tissue bank) or autologous grafts	Lausberg et al, 2006 Mirsadaee et al, 2006

Table 2. Applications of pericardium as medical devices.

1984; Moon & West; 2008), and heart valves (Ishihara et al., 1981; Schoen & Levy, 1999; Flanagan & Pandit, 2003; Vesely, 2005). Pericardial bioprostheses have also been described for the treatment of acquired cardiac pathologies, including postinfarction septal defects (David et al., 1995), reconstruction of mitral valve annulus (David et al., 1995a, 1995b) or outflow obstruction (Sommers & David, 1997).

Additionally, pericardium has also been used for the construction of bioprostheses in non-cardiac treatments such as patches for vaginal (Lazarou et al., 2005) or abdominal wall reparation (Limpert et al., 2009), dural repair (Cantore et al., 1987) or tracheoplasty (Dunham et al., 1994).

6. Conclusion

For clinical application, pericardial tissue has to be decellularized to prevent an immune responses or immune-mediated rejection of the pericardium. Various decellularization protocols have been largely reviewed here. The choice of the decellularization strategy has an impact on the mechanical properties, the scaffold pore size, the scaffold tissue integration and the development of long-term calcification. All these considerations should be carefully taken into account when designing new pericardial-based biomaterials. Currently, glutaraldehyde is the gold standard for pericardial treatment used in clinical practice. Nevertheless, it has important drawbacks including cytotoxic effects, prevention of host cell attachment, migration and proliferation (Huang-Lee et al., 1990), and a high propensity to calcify. Alternative treatments to replace or complement glutaraldehyde crosslinking of the pericardium have been investigated using other crosslinking reagents, decellularization, lyophilisation or coating with biopolymers (Nogueira et al., 2010). Despite many studies, it is still difficult to know which strategy to adopt regarding pericardial treatment. First, we do not have enough follow-up to permit evaluation of most of these alternatives and treatments. Second, every new treatment proposed is generally compared only to glutaraldehyde. It is thus not possible to classify these treatments by efficiency. Finally, the protocol for an optimal treatment depends largely on the final application targeted. In addition, there have been recent advances in tissue regeneration with the emergence of cell therapy and new pericardial treatments with cellular growth factors promoting recellularization (Chang et al., 2007). However, further improvements need to be achieved to transform these techniques into clinical applications. The use of autologous pericardium in cardiac valvular therapy is also a challenging alternative. Nevertheless, it still currently requires the development of local pericardial treatments aiming to favor the valvular remodelling. The understanding of current issues and the improvement of pericardial processing may have a huge impact for bioprothesis conception and patient safety.

7. References

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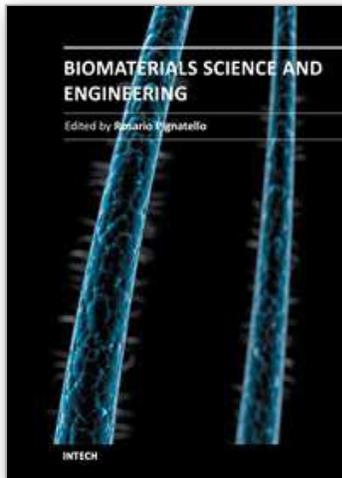
Biomaterials Science and Engineering

Edited by Prof. Rosario Pignatello

These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentials of different synthetic and engineered biomaterials. Contributions were not selected based on a direct market or clinical interest, than on results coming from very fundamental studies which have been mainly gathered for this book. This fact will also allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The book collects 22 chapters related to recent researches on new materials, particularly dealing with their potential and different applications in biomedicine and clinics: from tissue engineering to polymeric scaffolds, from bone mimetic products to prostheses, up to strategies to manage their interaction with living cells.

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