

METHOD OF MAKING CALCIFICATION-RESISTANT BIOPROSTHETIC TISSUE

RELATIONSHIP TO OTHER APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 07/689,652, filed on Apr. 23, 1991 now U.S. Pat. No. 5,368,608 issued on Nov. 29, 1994, as a continuation-in-part of Ser. No. 07/515,484 filed on Apr. 30, 1990, now abandoned, which in turn was a continuation-in-part of Ser. No. 07/176,789 filed on Apr. 1, 1988, now U.S. Pat. No. 5,094,661, issued on Mar. 10, 1992, all applications being assigned to the assignee hereof. The disclosure of the foregoing applications are incorporated herein by reference in their entirety.

GOVERNMENT RIGHTS CLAUSE

This invention was made with government support under Contract HL38118 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

This invention relates generally to materials which are resistant to *in vivo* calcification, and more particularly, to a method of preparing calcification-resistant biomaterials, such as bioprosthetic tissue, suitable for implantation in a living being.

More than 100,000 cardiac valve prostheses are placed in patients each year. Frequently, valve replacement surgery is the only means of treating cardiac valve disease. Currently used replacement valves include mechanical valves which may be composed entirely of a synthetic polymeric material such as polyurethane; bioprosthetic valves derived from bovine pericardium or porcine aortic valves; and aortic homografts.

Use of mechanical valves is frequently complicated by thrombosis and tissue overgrowth leading to valvular failure. Bioprosthetic heart valves have improved thrombogenicity and hemodynamic properties as compared to mechanical valve prostheses. However, calcification is the most frequent cause of the clinical failure of bioprosthetic heart valves fabricated from porcine aortic valves or bovine pericardium. Human aortic homograft implants have also been observed to undergo pathologic calcification involving both the valvular tissue as well as the adjacent aortic wall albeit at a slower rate than the bioprosthetic heart valves. Pathologic calcification leading to valvular failure, in such forms as stenosis and/or regurgitation, necessitates re-implantation. Therefore, the use of bioprosthetic heart valves and homografts has been limited because such tissue is subject to calcification. In fact, pediatric patients have been found to have an accelerated rate of calcification so that the use of bioprosthetic heart valves is contraindicated for this group.

Unfortunately, pathologic calcification also further complicates the use of synthetic vascular grafts and other artificial heart devices, such as ventricular assist systems, because it affects the flexibility of the synthetic polymers used to produce the devices.

The mechanism for pathological calcification of cardiovascular tissue is not fully understood. Generally, the term "pathologic calcification" refers to the undesirable deposition of calcium phosphate mineral salts. Calcification may be due to host factors, implant factors, and extraneous factors, such as mechanical stress. There is some evidence to suggest that deposits of calcium are related to devitalized

cells, and in particular, cell membranes, where the calcium pump ($\text{Ca}^{+2}-\text{Mg}^{+2}-\text{ATPase}$) responsible for maintaining low intracellular calcium levels is no longer functioning or is malfunctioning. Calcification has been observed to begin with an accumulation of calcium and phosphorous, present as hydroxyapatite, which develops into nodules which can eventually lead to valvular failure.

The preparation of bioprosthetic tissue prior to implantation typically includes treatment to stabilize it against subsequent *in vivo* enzymatic degradation, typically by crosslinking molecules, particularly collagen, on and in the tissue. Various aldehydes have been used for this purpose, including glyoxal, formaldehyde, and glutaraldehyde. Glutaraldehyde, however, is the agent of choice. In addition to fixing the tissue, glutaraldehyde is a good sterilizing agent and it reduces the antigenicity of the tissue. To date, glutaraldehyde is the only effective crosslinking agent for preparing tissues for implantation that can be used at physiologic pH under aqueous conditions. Unfortunately, glutaraldehyde is now known to promote calcification. There is, thus, a need in the art for a means of reversing the calcification-promoting effects of crosslinking agents such as glutaraldehyde. It would be particularly desirable to incorporate anti-calcification agents into existing protocols for preparation of clinical-grade biomaterials.

Non-aldehyde crosslinking agents have been investigated, such as polyepoxides (e.g., polyglycerol polyglycidyl ethers sold under the trademark Denacol by Nagasi Chemicals, Osaka, Japan), but there have been no conclusive studies demonstrating efficacy of polyepoxide cross-linked tissues *in vivo*.

Research on the inhibition of calcification of bioprosthetic tissue has primarily focussed on tissue pretreatment with either detergents or diphosphonate anticalcification agents. Detergent pretreatment with noncovalently linked detergents, such as sodium dodecyl sulfate (SDS), and a covalently bound detergent, such as amino oleic acid, have been demonstrated to be efficacious to materials exposed in circulating blood. However, both detergents and diphosphonates tend to wash out of the implanted bioprosthetic tissue with time due to blood-material interactions. Thus, these treatments merely delay the onset of the inevitable calcification process. Accordingly, there is also a need for a means of providing long-term calcification resistance for bioprosthetic heart valves and other implantable biomaterials or devices which are subject to *in vivo* pathologic calcification.

In addition, detergents disadvantageously affect the tissue, resulting in a diminution of the collagen denaturation temperature, or shrink temperature (T_s), which is an important measure of material strength, durability, and integrity. In some cases, use of detergents results in local toxicity. There is, thus, a need for an effective method of imparting anti-calcification properties to bioprosthetic tissues which is not accompanied by the deleterious effects of detergents.

Further, all of the foregoing techniques still result in some degree of pathologic calcification *in vivo* as measured by calcium content of explanted specimens. There is, therefore, a need for a treatment that results in a greater level of calcification inhibition.

The use of alcohols in biomaterial treatment protocols is well-known, but is typically limited to its use as a solvent and/or sterilizing agent. For example, alcohol has been used in sterilizing rinses and for storage solutions. However, there has never been any teaching or suggestion that ethanol has any effect on prevention of pathologic calcification. It would be advantageous to use this well-known compound in existing protocols for rendering bioprosthetic tissue calcification-resistant.