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Biomaterials for Injectable Therapeutic Implants

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Abstract: Injectable biomaterials that have the ability to form semi-solid implants *in situ* are of keen interest for therapeutic applications. The materials may be used to fill in pathological vascular spaces or be designed to possess functional properties such as being radiopaque for an improved visibility during image-guided minimally invasive interventions, or induce a localized biological activity. Among the variety of solidification principles that may be used to produce implants *in situ*, the precipitation of water-insoluble polymers driven by solubility changes shows some particular features that may be valuable for specific therapeutic applications. This paper reviews some of the applications of these implant-forming biomaterials in interventional radiology, urology, and oncology.

Keywords: Embolization · Injectable biomaterials · Interventional procedures · Oncology · Polymeric implants · Urology

1. Introduction

Polymeric biomaterials can be designed to form a semi-solid implant *in situ* once injected in the body. This implant may allow for a localized therapy with reduced patient immobilization and health costs when compared with surgical approaches. *In situ* forming implants have been used for the treatment of pathological blood vessels, tumors, tissue bulking or to release drugs. The delivery of the implant can be achieved either by direct needle puncture or by using endovascular catheterization. As for the latter case, advances in the field of image-guided minimally invasive delivery techniques have spurred the search for biomaterials compatible with fluoroscopy, computerized X-ray tomography or magnetic resonance imaging.

We may distinguish different physicochemical principles to achieve the required *in situ* phase transition following injection: (i) cross-linking or polymerization of monomers or oligomers, (ii) phase change driven by pH or ionic concentration changes, and (iii) precipitation following solvent exchange. Formulations of the two former categories have been thoroughly reviewed elsewhere [1–3]. This paper focuses on injectable biomaterials developed in our labs for application in interventional radiology, oncology and urology, with a special interest for precipitating implants.

The injectable precipitating formulations are based on water-insoluble preformed polymers dissolved in water-miscible organic vehicles. Once injected into an aqueous environment, the solubility changes driven by the solvent exchange induces the precipitation of the polymer. A cast that conforms to the contours of irregularly shaped vascular defects or body cavities is thus formed. In contrast to *in situ* polymerizable materials (i), no initiator, monomers or free radicals are released, avoiding potential acute toxicity or carcinogenicity. Due to the rapid solvent exchange, a skin is first formed at the interface between the forming implant and the surrounding aqueous environment. This skin advantageously prevents leakage of the polymer into the circulating blood or surrounding tissues, a specific feature of precipitating formulations that allow for a more precise control of the polymer distribution into the body cavity to be filled.

The expected therapeutic effect may result from the implant itself, as for instance in the filling of an aneurysm space that excludes it from blood circulation and consequently reduces the risk of hemorrhage. In contrast to these inert materials, bioactive implants can contribute to tissue remodeling or induce a therapeutic effect by presenting or releasing active substances, as for instance with drug-loaded delivery systems.

2. Space-filling Implants

2.1. *Embolization Materials*

Endovascular approaches have become widely accepted alternative treatments for the occlusion (embolization) of intracranial aneurysms and arteriovenous malformations (AVMs). Despite the unquestionable benefits of metallic coil embolization, some limitations arose such as the difficulty to achieve a complete aneurysm occlusion, or the reformation of blood channels in embolized arteriovenous malformations. Alternative liquid embolics were proposed for their ability to fill in completely these vascular defects. Among the various liquid formulations evaluated for embolization, a precipitating cellulose acetate polymer solution in dimethyl sulfoxide (DMSO) was first proposed [4] that allowed for the effective embolization of aneurysms [5] and AVMs [6] in clinics. Mild inflammatory reaction and absence of chronic granuloma

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were reported; the implant induced a local thrombosis and could ultimately develop an endothelial layer over the aneurysm. A non-adhesive ethylene-vinyl alcohol copolymer diluted in DMSO could also embolize AVMs [7–9] and aneurysms [9–11]. A formulation based on the same material (Embolyx™ or Onyx™) was shown to embolize efficiently patient AVMs [12][13], intracranial aneurysms [14] and liver tumors [15]. This product received the CE mark for peripheral vascular applications and brain tumors. A reported drawback of this polymer solution is its slow or poor solidification that can lead to the migration of the embolic agent into the parent artery [16]. Poor radiopacity and radiopacifier sedimentation were also mentioned. Although these results demonstrated the ability of liquid embolics to fill aneurysmal defects in a more efficient way than coils, their clinical use is still hampered by the difficulty to confine the implant to the targeted aneurysm, and by the established angiotoxicity of DMSO.

A key issue for precipitating liquid embolics is the choice of the organic solvent used as a vehicle for the polymer. While solvent systemic toxicity is still a controversial issue for embolization implants that have a typical volume smaller than a few cm^3, local toxicity can be a major drawback. As for intra-arterial infusion, DMSO angiotoxicity occurs under the form of vasospasms, damage to aneurysm wall and angionecrosis that lead to a poor embolization outcome [17]. These effects can however be reduced to a clinically tolerable level using a very slow rate of infusion [18]. Another potential drawback of DMSO for intravascular use is its hemolytic activity. Solvent hemolytic activity has been measured *in vitro* on red blood cells [19] and the hemodynamic activity measured as the intra-arterial pressure change following intravenous solvent infusion. Pharmaceutical excipients showing a reduced hemolytic and hemodynamic activity (Table) have thus been proposed as a DMSO replacement, such as Glycofurol 75, N-methyl pyrrolidone (NMP), Solketal or isosorbide dimethyl ether (DMI), which has the lowest potential for hemolysis. Injectable biomaterials based on these alternative vehicles [20] could thus offer a safer embolization and an improved control of implant delivery.

Table. Acute toxicity, hemolytic and hemodynamic activities of selected organic vehicles for endovascular precipitating implants

	Intravenous LD 50, rat[a] [g/kg]	Hemolytic activity[b]	Hemodynamic activity[c]
Dimethyl sulfoxide	5.4	+++	+++
N-methyl pyrrolidone	2.3	++	+++
Glycofurol	3.8	++	+
Isosorbide dimethyl ether	>5	+	+
Solketal	6.7	++	++
Ethyl lactate	–	+++	+++
Ethanol	1.4	++	++

[a]Acute toxicity from the Registry of Toxic Effects (RTECS); [b]Hemolytic ativity *in vitro* at 1%, 5% and 10% solvent concentration in water: +++ strong hemolysis at 1%, ++ strong hemolysis at 5%, + weak hemolysis at 1%; [c]Hemodymanic activity on sheep: +++ strong hemodynamic activity corresponding to an average relative arterial pressure increase of >10% following solvent injection, ++ >5%, + <5%

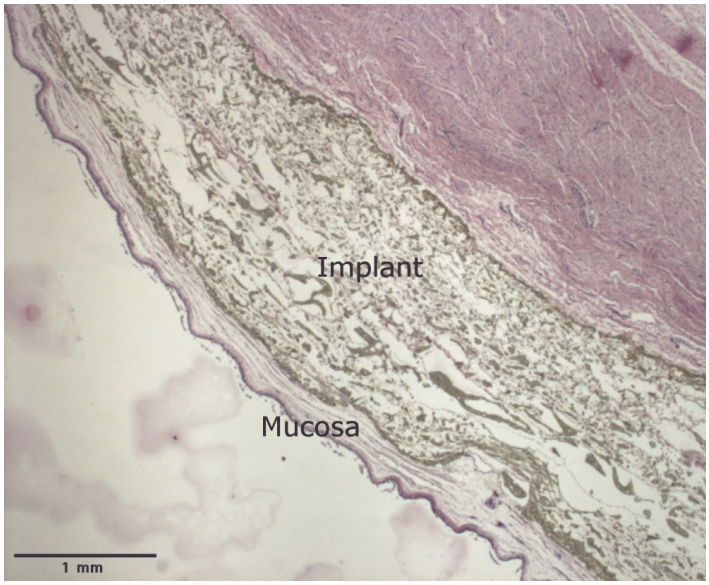

Fig. 1. Histological section of a urethra after submucosal injection of a bulking agent based on a medical-grade polyurethane solution in NMP. The implant preserved the mucosal and subepithelial layers.

2.2. *Aqueous-based Embolization Materials*

In addition to polymerizable and precipitating systems, phase transitions driven by ionic concentration or pH change are attractive due to the absence of solvent and potentially toxic polymerization by-products. In the field of embolization, ionically crosslinkable polysaccharides such as alginate could embolize swine AVMs [21]. Enzymatically crosslinkable fibrin glue, a FDA-approved hemostatic agent, could efficiently embolize hemangiomas [22] and meningioma [23]. Thermally responsive polymers, such as poloxamers [24] or poly(n-isopropylacrylamide) [25], were also proposed for embolization. Although effective on the short term and generally displaying a good biocompatibility, these hydrogels have not proved yet superior to poly(ethylene-co-vinyl alcohol) precipitating formulations due to their rapid degradation or poor handling. Further developments towards increased *in vivo* durability and formulation stability are still required.

2.3. *Tissue Bulking*

Besides endovascular applications, endoscopy allows the delivery of space-filling implants for tissue bulking, *i.e.* increasing tissue volume through the injection of a semi-solid biomaterial. An example is given by stress urinary incontinence, a common problem that can be treated by endoscopic injection of a urethral bulking agent (UBA) under urethroscopic guidance. The injected UBA improves mucosal coaptation, which in turn increases urethral closure pressure and hence continence. Although collagen has proven efficient for the treatment of urinary incontinence, its effects are limited in time, thus requiring repeated injections. In order to avoid premature degradation, various suspensions of non-degradable microspheres [26] have been proposed that, despite their efficient bulking effect, tend to migrate to distant organs [27][28]. Alternatively, experimental precipitating bulking

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agents have been developed in our group (Fig. 1) [29], that may not undergo migration and could possibly provide a durable recovery of continence.

3. Functional and Bioactive Implants

3.1. *Radiopaque Polymers*

A key function of the biomaterials designed for endovascular delivery is their visibility under common imaging techniques to allow for a safe delivery. Visibility under X-ray imaging such as fluoroscopy or computerized tomography requires radiopaque formulations. Most of the above-mentioned liquid embolics are made radiopaque by the adjunction of a solid contrast agent such as tantalum, tungsten or bismuth trioxide. Entrapment of the radiopaque agent into the polymer is however not ensured, so that phase separation and/or leaching of those particles could hinder the clinical follow-up and lead to toxic effects. In addition, sedimentation of the insoluble agents can impede a precise visualization under CT. Polymers with bound radiopaque elements may be an answer to these concerns. The first approach consists in grafting a radiopaque element (generally iodine) onto a monomer before polymerization. This approach led to radiopaque polymers that demonstrated biocompatibility [30–32]. However, radiopaque liquid embolics require high molecular weight polymers to precipitate into a cohesive polymer mass, and high iodine content to ensure a good visibility. A biodegradable polyurethane-based radiopaque solution meeting these requirements demonstrated an efficient embolization of pig liver [33]. The second approach consists in grafting radiopaque elements onto high molecular weight preformed polymers. Cellulose, known for its biocompatibility, was grafted with iodinated groups to produce highly radiopaque polymers that are soluble in DMSO and other organic solvents. Appropriate embolization was demonstrated in sheep [34]. Similarly, poly(vinyl alcohol) was iodinated [35] and used to embolize surgically created aneurysms (Fig. 2) with a mean aneurysm occlusion degree of 96%, comparable to Onyx™, a commercial product radiopacified with tantalum [36]. Although these studies have demonstrated the value of radiopaque embolics, further characterization of polymer stability and biocompatibility are still required for a clinically applicable radiopaque embolic agent.

3.2. *Sclerosing Embolics*

Bioactive liquid embolics that induce a local sclerosis leading to blood vessel occlusion may also be advantageous to treat AVMs. Sclerosis can be obtained through the use of a solvent such as ethanol, or a surfactant. Aqueous solutions containing up to 40% ethanol have been used to confine the sclerosis to intimal vessel layers [37] and preserve surrounding tissues. Poly(vinyl acetate) (PVAc), when partially hydrolyzed, may be dissolved in such ethanol concentrations. The efficiency of hydrolyzed PVAc was demonstrated for renal embolization in pigs [38] and in clinics [39]. A commercially available protein solution containing ethanol, Ethibloc™, has been used for AVM and tumor [40] embolization. Although some of these ethanol-based solutions may be relevant in clinics, the risk of injury to adjacent normal tissues is still a concern; some authors recommend limiting the use of ethanol to small organs such as kidneys.

3.3. *Bioactive Implants*

In order to induce a specific response of the tissue surrounding the implant, bioactive substances may be grafted onto or released by the polymer matrix. For instance, cell adherent polymers may increase tissue remodeling and accelerate aneurysm healing. With this assumption in mind, an ethylene vinyl alcohol copolymer was grafted with ProNectin-F, a polypeptide containing copies of the RGD cell attachment ligand [41]. The polymer was used to embolize surgically created aneurysms in rats. ProNectin-F-bearing polymer could enhance fibroblast proliferation around the implant. Likewise, basic fibroblast growth factor could promote aneurysm healing [42], a strategy that may lead to a more complete and permanent aneurysm occlusion, and potentially to an improved clinical outcome.

Incorporation of a drug into a precipitating polymer injected at a localized site offers the advantages of a simple application and a localized drug delivery [43]. Such drug depots have been extensively studied, for instance for the local delivery of antitumor agents [44], tumor necrosis soluble factor [43], bone morphogenetic proteins [45] or genes [46]. A mild inflammatory response to subcutaneous and intramuscular injections of biodegradable polymers dissolved in N-methyl pyrrolidone or dimethyl sulfoxide in rhesus monkeys was reported, similar to that obtained with biodegradable polymers [47]. However, contradictory reports of myotoxicity raised concerns about solvent local effects [48]. The burst release may also be difficult to avoid using this approach [3]. Nevertheless, a site-specific drug-delivery system based on a biodegradable poly(α-hydroxy acids) dissolved in NMP was approved by the FDA (Atrigel® Implant Drug Delivery Technology, Atrix Laboratories, Fort Collins, CO) and currently used to deliver leuprolide acetate for prostate cancer treatment. Alternative aqueous mixtures of solvents [49] or solvents such as benzyl or ethyl benzoate [50] have also been proposed, in particular to improve implant biocompatibility. Still, the issues of protein stability in the formulations and reduction of burst release deserve further investigations.

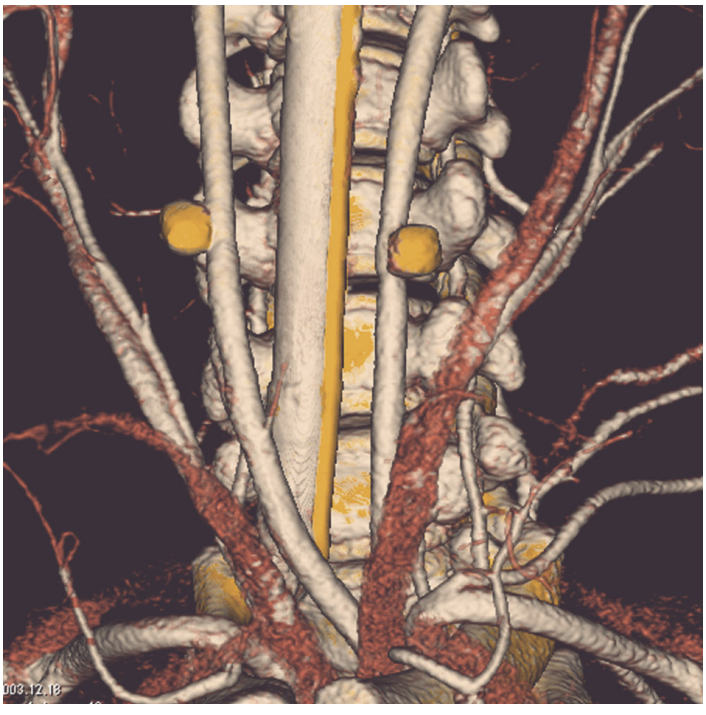

Fig .2. Angiographic 3D reconstruction showing the complete occlusion of surgically created porcine aneurysms embolized with a radiopaque polymer

4. Conclusion

Developments in the design of precipitating biomaterials that can form implants *in situ* lead to various novel applications, which in some cases have reached clinical practice. Although the use of organic

vehicles limits the implants to small volumes and the intra-arterial injection to slow rates, new vehicles may broaden the range of use. The implant chemistry and formulation depend critically on the envisioned application, more specifically on the delivery route, tissue response to the implant, and the need for a biological implant activity. As for endovascular access, the skin-forming ability of precipitating materials when in contact with blood remains a distinctive and attractive feature. The developments in radiopaque polymer synthesis may improve implant visibility under common imaging techniques, thus contributing to safer delivery and follow-up. Although still experimental, bioactive materials that may help to control the response of the tissue surrounding the implant hold promises in various fields of minimally invasive treatments.

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