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# Fabrication of tannic acid/poly(*N*-vinylpyrrolidone) layer-by-layer coating on Mg-based metallic glass for nerve tissue regeneration application

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A B S T R A C T

For improving recovery rates and functional outcomes in large nerve defects, a nerve guide conduit, in addition to topographic, physical and chemical cues should provide contact guidance and adequate mechanical support for cell migration and axon outgrowth. Among biomaterials, magnesium (Mg) metal has potential to support nerve regeneration owing to its electrical conductivity, biodegradation and ability to be formed into wires, filaments and ribbons. However, rapid degradation of magnesium can pose a challenge. Mg-based metallic glasses with desirable features including favorable biocompatibility, proper biodegradation and good mechanical properties are a good alternative to crystalline Mg alloys. This study investigates the biocorrosion and biocompatibility of Mg-Zn-Ca metallic glass ribbon with $Mg_{70}Zn_{26}Ca_4$ composition. For controlling biocorrosion, layer-by-layer coating of tannic acid/ poly(N-vinylpyrrolidone) was applied on $Mg_{70}Zn_{26}Ca_4$ ribbon and characterized by SEM and FTIR. Immersion and potentiodynamic polarization test results indicated that coating significantly improved the corrosion resistance of $Mg_{70}Zn_{26}Ca_4$. Schwann cells were selected for the cytocompatibility evaluation of samples due to their key role in peripheral nerve regeneration and ability to repair spinal cord injuries. The MTT assay and cell morphology results showed good biocompatibility for $Mg_{70}Zn_{26}Ca_4$ metallic glass as a promising candidate for nerve regeneration and implantable nervous prosthetic devices.

## 1. Introduction

Nerve injuries are debilitating conditions that cause loss of sensory and motor functions and result in significant costs to both individuals and society [1,2]. In comparison with the central nervous system (CNS), the peripheral nervous system (PNS) has greater spontaneous recovery capacity [3,4]. After PNS injury, both the axon and the myelin sheath in the distal stump degenerate and macrophages and Schwann cells (SCs) act to remove myelin and axon debris [5,6]. After the removal of debris, proliferating Schwann cells align and form longitudinal bundles surrounded by basal laminae, known as Bands of Büngner, through which axons regenerate [5,7]. Schwann cells further secrete a range of neurotrophic factors and cell adhesion molecules, all of which promote axonal regeneration [8,9]. For peripheral nerve defects where suturing is not possible, autografting is the gold standard for nerve repair [10]. However, limitations such as donor site morbidity, limited availability and size mismatch for autografts, and risk of immunorejection for allografts and xenografts, have limited their use [11,12]. Nerve guidance conduits (NGCs) are another approach that have gained popularity over the years [4,13]. Although, in peripheral nerve gaps longer than 2–4 cm, regeneration through conduits is poor and full recovery of nerve function is not often achieved [10,14,15]. A reason for this problem is a lack of support to allow the regenerating axons to cross the gap [16]. Speculation and experimental evidence suggest that NGC capabilities could be improved by adding internal (preferably linear) scaffolding materials that provide physical support for axons to cross nerve gaps [17,18]. Today, multifunctional NGCs including physical or chemical guidance cues and longitudinal topography have attracted great attention [19]. In recent years, some studies have described methods to make nerve scaffolds that incorporate oriented structures to provide effective support for axons to cross from the proximal stump to the distal stump, for example: aligned nanofibers [12], microgrooved surfaces [20], multichannel [21] and ice-templated structures [17].

Among biomaterials, synthetic or natural polymers such as collagen, chitosan, silk, agarose, alginate, poly(lactic-*co*-glycolic acid) and polycaprolactone have been included in most nerve regeneration studies [22]. However, because most polymers have no intrinsic electrical and chemical cues, incorporation of molecules and growth factors is required to achieve full recovery of motor and sensory functions. Also, studies have revealed potential application of bioactive ceramics in

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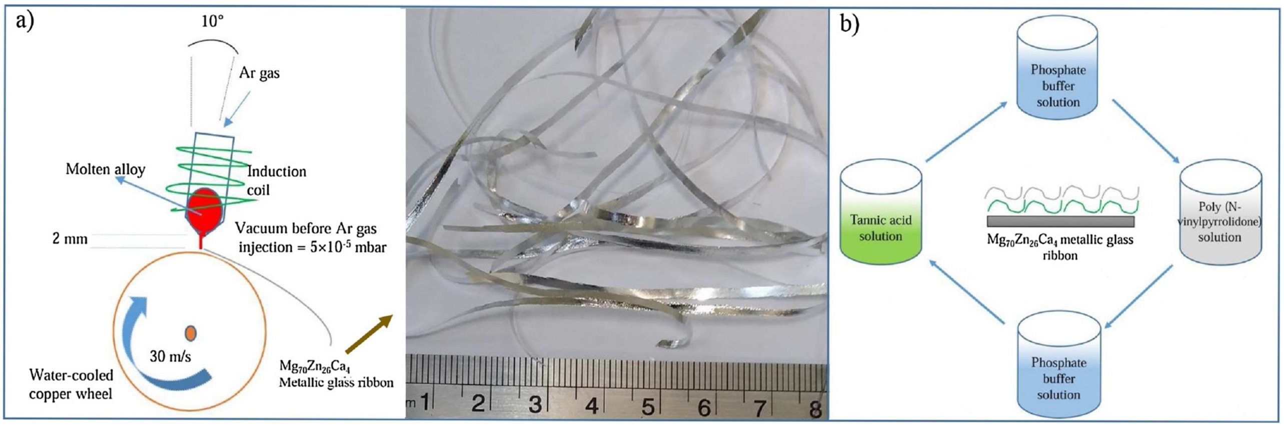


**Fig. 1.** (a) Schematic of melt-spinning technique with some parameters used for producing $Mg_{70}Zn_{26}Ca_4$ metallic glass ribbon and (b) schematic of layer-by-layer assembly technique for preparation of (TA/PVPON) coating on $Mg_{70}Zn_{26}Ca_4$ metallic glass ribbon.

nerve regeneration [23–25]. It is reported that axonal regrowth across a 0.5 cm interstump gap in Bioglass® 45S5 was comparable with that seen using an autograft [23]. Also, phosphate [24] and borate-based glass [25] fibers' potential in nerve regeneration and their roles in axon directional guidance was investigated. Despite significant recent progress, studies using metals for nerve regeneration have not been pursued seriously. In 2014, Vennemeyer et al. [26] reported biocompatibility of pure Mg wires by repairing sciatic nerve gap injuries. Electrical conductivity, alloying ability with effective elements, and magnesium salt clinical applications [27], show the high potential of biodegradable magnesium in both nerve devices and nerve regeneration. However, rapid degradation of magnesium metal can be a major challenge for long nerve gaps [28]. Among Mg alloys, Mg-based metallic glasses (MGs) have emerged as promising candidates for biomedical applications in recent years [29]. Unlike their crystalline counterparts, MGs are single phase and chemically homogenous [30,31]. Also, the absence of microstructural defects such as grain boundaries, dislocations and precipitates can slow down the degradation rate, prevent rapid strength loss and extend the service life for biodegradable applications [32,33]. Moreover, it is confirmed that Mg-based MGs show less hydrogen gas pockets compared with crystalline alloys generated by the corrosion reaction [34]:

$$Mg + 2H_2O \rightarrow Mg^{2+} + 2OH^- + H_2.$$

Among Mg-based MG compositions, the Mg-Zn-Ca system [29] is a favorite choice for bio-implant applications because it contains the essential elements Zn and Ca for humans. For example, in nerve tissue, calcium ions regulate motility of the axonal growth cone and the guidance of growth cone extension, and zinc plays a role as a signaling substance [35]. However, for larger gaps, a nerve guidance conduit is needed to simultaneously supply contact guidance and a proper microenvironment, and must maintain its integrity for nerve regeneration [36]. For this purpose, among various methods for coating magnesium metal, dip coating is a cost-effective option [37]. Considering both enhancing the corrosion resistance of Mg alloy (without altering the bulk properties) and biocompatibilty [38], polymeric coatings are more popular. Also, it has been demonstrated that multilayer coatings prepared via a layer-by-layer technique have anti-corrosion and self-healing properties on the substrate [39]. Researchers have demonstrated tannic acid (TA) as a corrosion inhibitor for metals [40]. TA belongs to a group of hydrolysable tannins and contains digalloyl ester groups connected to a glucose core, and its antibacterial and antioxidant properties are noteworthy [41]. It is reported that TA can form hydrogen bonds with neutral polymers and cover the range of pH values, from physiological environment to more basic values [42].

In this study, a ribbon form of Mg-based metallic glass in Mg-Zn-Ca system was prepared using melt-spinning technique, and its potential application for nerve tissue regeneration was investigated. Moreover, TA/ poly (*N*-vinylpyrrolidone) (PVPON) layer-by-layer assembly film was prepared on the ribbon surface by dip coating. TA/PVPON films were characterized by FTIR and SEM, and the film's effect on the ribbon biodegradation behavior was evaluated by immersion and potentiodynamic polarization tests. Because Schwann cells play an important role in peripheral nerve regeneration, initial observations of Mg-Zn-Ca metallic glass ribbon biocompatibility was studied with Schwann cells by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay and cell morphology by scanning electron microscope.

## 2. Materials and methods

### 2.1. Materials

A master alloy with the nominal composition of $Mg_{70}Zn_{26}Ca_4$ (in atomic percentage, at.%) was prepared in an induction melting furnace under the protection of argon and $SF_6$ gas atmosphere. For preparing layer-by-layer coating, TA (Mw, 1701.20 g/mol) and PVPON (Mw, 360,000 g/mol) were purchased from Sigma–Aldrich. 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide solution, Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin–EDTA and penicillin-streptomycin were supplied by Sigma–Aldrich.

### 2.2. Preparation and characterization of Mg-based metallic glass ribbon

$Mg_{70}Zn_{26}Ca_4$ MG ribbon was fabricated using a melt-spinning machine in an argon atmosphere from the master alloy to produce ribbon with a thickness and width of approximately 40 µm and 2 mm, respectively. Fig. 1(a) shows a schematic of the melt-spinning technique with some parameters used for producing $Mg_{70}Zn_{26}Ca_4$ MG ribbon. X-ray diffraction (X'Pert MRD model, PANalytical company, Netherlands) was used to verify the amorphous structure of melt-spun $Mg_{70}Zn_{26}Ca_4$ ribbon using Cu-$K_\alpha$ radiation at 45 kV and 40 mA from 10° to 90°.

### 2.3. Preparation of layer-by-layer (LbL) coating

The prepared ribbons were used with a length of 6 mm for LbL coating of (TA/PVPON). The surfaces of the samples were gently polished with SiC sandpaper of #2500 and then ultrasonically cleaned in ethanol (70%) for 4 min and rinsed with distilled water, followed by drying in air. The LbL films were applied by dip coating. Briefly, the bilayer of TA (0.25 mg $mL^{-1}$, pH 7.3, in 0.01 M PBS) and PVPON (0.25 mg $mL^{-1}$, pH 7.3, in 0.01 M PBS) were deposited alternatively with ~30 s wash with 0.01 M PBS. Fig. 1(b) shows a schematic of the LbL technique for (TA/PVPON) coating preparation on $Mg_{70}Zn_{26}Ca_4$