Medicinal Mushrooms in Wound Healing Part II

Schizophyllum commune
("Splitgill")

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Fungi have traditionally been utilized as wound-healing technologies by cultures all over the world. The many reports of early peoples utilizing fungi for wound healing have opened many avenues for scientific questioning.

Can these healing properties be replicated in a lab setting? Some mushrooms, including puffballs, were used at various maturity levels but mostly as mature gleba or spores; is maturity level associated with degree of wound-healing activity? Can we identify what molecules contribute to healing properties? The answer to many of these questions is, in general, yes.

Lycoperdon species ("Puffball")

Before getting into it, I want to go over a few concepts. One word used a lot in studies involving living organisms is polysaccharides, which are sugar molecules connected in a long chain. Polysaccharides are ubiquitous in nature, representing some of the most common and necessary compounds for life such as energy-storing carbohydrates and structural compounds like cellulose and lignin in plants or chitin in fungi and other organisms. When investigating substances for wound-healing properties, a place many researchers start is with cell cultures, running experiments like growing skin cells in a petri dish, disturbing them in some manner such as scratching them, and then measuring parameters related to the healing process such as cell migration or levels of gene expression.[1] Some cell studies use different types of cells, and some obtain other measures like microbial resistance. Certain experiments determine parameters, like antioxidant activity using chemical assays (antioxidants are thought to promote wound healing by helping control oxidative stress).[2] Another common route for probing for pro-healing potential is by inducing reproducible injuries in animal models such as mice or rats and measuring such parameters relevant to the healing process as levels of inflammatory signals, speed of wound closure and degrees of tissue remodeling (blood vessel formation, collagen deposition, ect.).[3] In the context of natural products like fungi, these studies often involve some sort of extraction process to isolate certain groups of molecules prior to application to the wound in order to single out which molecules are responsible for which properties.

The humble puffball has quite the history as a medicinal mushroom and researchers have taken notice. Two studies out of the University of Belgrade report on puffball species, specifically investigating whether the maturation of the fruitbody had significant effects on levels of compounds by measuring those compounds using analytical techniques and determining biological activity through chemical assays. One study compared mycelium, young fruitbody, and mature fruitbody (the powdery, sporey mass that is the gleba of a mature puffball) of *Bovistella utriforme* and found increased antioxidant activity in extracts from gleba compared to younger fruitbodies and mycelium, as well as increased levels of certain bioactive molecules like phenols and free ergosterol, among others.[4] In a separate study, *Lycoperdon excipuliforme* and *Lycoperdon pratense* extracts from young and mature fruitbodies were screened for potential medicinal skin-care compounds, and the researchers found higher concentrations of compounds with known antioxidant properties in the mature gleba.[5] These researchers concluded that the process of the puffball transforming from firm fruitbody to sporey gleba leads to production of certain medicinal molecules and suggested the maturation process may even serve to preserve these compounds. In both studies, the immature puffballs were slightly more effective than their mature counterparts in terms of antimicrobial activity; however, mature puffballs still exhibited an antimicrobial effect. A 2022 study in China investigated puffball spores as a wound-healing agent in a diabetic-rat model (a pertinent model, as diabetes and related metabolic syndrome are global epidemics and adversely affect the wound-healing process, increasing the likelihood that a wound will be chronic). This study reported accelerated wound-closure time in spore-treated rats compared to untreated controls and noted improved tissue remodeling, improved vascular signals, reduced inflammation and increased antioxidant activity.[6] A further example of modern researchers leveraging age-old techniques comes from scientists in Mongolia who combined a protein called calvacin, derived from the giant puffball *Calvatia gigantea*, with a bioactive plant extract to produce an effective burn-healing agent that improved healing in rats (these researchers note the traditional use of this mushroom in wound management in Mongolian medicine).[7]

The vast assortment of fungi with wound-healing potential in ethnomycological reports is enough to make you wonder, do all fungi have what it takes to improve the wound-healing process? It might be a stretch to make such a claim; however, several familiar classes of compounds found in the cell walls of all fungi are quickly becoming some of the most popular tools used by researchers to tackle problems related to wound healing. One such compound is chitin, that compound that you hear about when being told always to cook your mushrooms before eating them. Chitin makes up a significant portion of the cell walls in fungi and has biodegradable, hemostatic (i.e., it stops bleeding), antimicrobial properties and, importantly, its structure is a good mimic of the extracellular matrix in animals (in other words, it is generally highly biocompatible in humans and has many similar properties as our tissues).[8,9] Chitin is relatively easy to extract; however, it is hard to dissolve and thus difficult to fine-tune for biomedical applications.

This brings us to our second powerful fungal polysaccharide, chitosan. Chitosan is a derivative of chitin and is found naturally in many fungi, although generally not in as high quantities as chitin. Chitosan has the healing properties of chitin with a few major upgrades: (1) it is easy to dissolve and manipulate into all sorts of nanofibrous scaffolds, grafts, gels, powders or membranes; and (2) it is simple to modify chemically and attach molecules with additional bioactive properties.[8] Chitin and derivatives like chitosan are the only positively charged naturally occurring biopolymers which, when applied to a bleeding wound, provide benefits that include promoting coagulation by attracting and adsorbing red blood cells and platelets.[10,11] Chitosan is popular in the biomedical engineering space, with many current and evolving applications in tissue regeneration and wound healing, among other uses. In the Biomedical Engineering department at the University of Memphis, researchers are working on innovative applications for chitosan such as bone scaffolds for craniofacial surgery,[12] guided bone generation membranes,[13] or electrospun membranes for skin wound healing.[14] Beyond fancy tissue-engineering applications, chitosan is the major component in FDA-approved hemostatic bandages,[15] gauzes,[16] powders and more (you can even find some on Amazon). Most chitin used to derive chitosan for biomedical purposes comes

Tremella fusiformis ("White Jelly Fungus") from crustaceans; however, chitin derived from mushrooms is a simpler process that is relatively underexplored in biomedical applications.[17]

Continuing the trend of potent pro-healing polysaccharides is a familiar face in the medicinal mushroom realm, the beta-glucan. Beta-glucans are abundant in fungal cell walls and possess a particular penchant for modulating the immune response. Beta-glucans applied topically are reported to have antioxidant, anti-inflammatory and antimicrobial effects. Like chitin and chitosan, beta-glucans are recognized as an excellent mimic of the mammalian extracellular matrix and can be manufactured as hydrogels, nanofibers and membranes for advanced wound-healing applications.[18] A 2019 study in Korea prepared wound dressings from beta-glucans isolated from splitgill (*Schizophylum commune),* which accelerated healing in mice,[19] and a similar study from the same research group compared wound-healing effects of beta-glucans from splitgill, black yeast, barley, and a protist and found the splitgill polysaccharides to be the most effective at promoting healing in cell and mouse experiments.[20] A study out of Brazil reported that beta-glucans from the birch polypore (*Piptoporus betulinus)* promoted cell migration in a cell-scratch assay.[21]

The many wound dressings developed with healing polysaccharides accelerate healing in many cases where traditional wound dressings like pads or gauze might fail (due to need for periodic dressing changes and given the environment they foster is less conducive to healing),[19,22] and are being explored as hemostatic agents in many conditions such as traumatic injuries[16,23] (including internal bleeding and surgeries)[24] and other conditions where limiting bleeding is important and difficult to control.[25] It is essential to keep in mind that polymers like chitin, chitosan,[17] and beta-glucans are not individual compounds but rather vast groups of related compounds with varying sizes, branching, structures and attachments, with each variation affecting potential biological activity. Given the natural variation of these polymers and myriad of potential applications, there is plenty of research left to be done.

As spores and mushrooms are composed of the powerful polysaccharides discussed above, they have many of the same properties. Ancient peoples around the world used spores and powdered fungal fruit bodies as hemostatic, wound-healing agents. Using such fine particles provided several important benefits in the high surface area for increased interaction at the wound surface[26,27] and ability to cover highly irregular, deep wound surfaces.[28] Similar to the ancient use of fungal powders, modern researchers recognize the power of powders with chitosan and other polysaccharides researched and utilized today in treating traumatic civilian and combat wounds.

Auricularia species ("Wood Ear")

Beyond the puffballs mentioned earlier, some fungi with ethnomycological reports for improving outcomes after injury[29] have been researched and reported to benefit healing processes, including King Alfred's cakes or *Daldinia concentrica* (crude extract improved wound healing in rats)[30] and tiger's-eye mushroom (*Lignosus rhinocerus)* (polysaccharide extract improved healing in gastric cell study).[31] Additionally, many mushrooms more commonly associated with medicinal potential have been researched and found to have wound-healing properties: lion's mane (*Hericium erinaceus;* crude extract accelerated wound closure in rats);[32] wood ear (*Auricularia auricula*; polysaccharide extract improved healing in excised pig ear cells);[33] polysaccharide extract improved healing in cell studies),[34] snow fungus (*Tremella fuciformis;* polysaccharide extract promoted healing in excised pig ear cells);[33] reishi (*Ganoderma lucidum;* polysaccharide extract enhanced wound healing in rats;[35] polysaccharide extract improved healing of gastric ulcers in rats;[36] spore oil improved burn healing in mice);[37] hemlock reishi (*Ganoderma tsugae;* extracted chitin improved wound healing in cell studies),[38] and *Ganoderma ambionense* (polysaccharide extract improved wound healing in cell studies).[39] Several choice edible mycorrhizal species have been screened for wound-healing potential, including *Cantharellus*,[40] and matsutake (*Tricholoma matsutake)*,[41] with separate studies finding extracts of each to accelerate healing in rodents. Two studies by a research group in Japan even report improved wound healing upon consumption of cauliflower mushroom (*Sparassis crispa)*. In one study, oral administration improved healing in diabetic mice[42] and in another both oral and topical beta-glucan administration independently sped up healing in diabetic mice.[43]

Fungi provided our most ancient ancestors with food, medicine and opportunity; today they inspire cutting-edge research that promises to improve health for future generations. Building on top of the strong foundation laid by earlier peoples, researchers have been able to verify many healing properties of medicinal mushrooms, with some mushroom derivatives being crafted into advanced biomedical biomaterials. The combined body of ethnomycological reports and peer-reviewed research make a strong case for further exploration of fungi as agents for wound healing.

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