Review Article

Serum albumin as a local therapeutic agent in cell therapy and tissue engineering

Dénes B. Horváthy*
Melinda Simon
Charlotte M. Schwarz
Mariana Masteling
Gabriella Vácz
István Hornyák
Zsombor Lacza

Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary

Abstract

Albumin is a major plasma protein that has become ubiquitous in regenerative medicine research. As such, many studies have examined its structure and advantageous properties. However, a systematic and comprehensive understanding of albumin's role, capabilities and therapeutic potential still eludes the field. In the present work, we review how albumin is applied in tissue engineering, including cell culture and storage, *in vitro* fertilization and transplantation. Furthermore, we discuss how albumin's physiological role extends beyond a carrier for metal ions, fatty acids, pharmacons and growth factors. Albumin acts as a bacteriostatic coating that simultaneously promotes attachment and proliferation of eukaryotic cells. These properties with the combination of free radical scavenging, neutrophil activation and as a buffer molecule already make the albumin protein beneficial in healing processes supporting functional tissue remodeling. Nevertheless, recent data revealed that albumin can be synthesized by osteoblasts and its local concentration is raised after bone trauma. Interestingly, by increasing the local albumin concentration *in vivo*, faster bone healing is achieved, possibly because albumin recruits endogenous stem cells and promotes the growth of new bone. These data also suggest an active role of albumin, even though a specific receptor has not yet been identified. Together, this discussion sheds light on why the extravascular use of the albumin molecule is in the scope of scientific investigations and why it should be considered as a local therapeutic agent in regenerative medicine.

Keywords: *serum; albumin; tissue engineering; regenerative medicine; bone*

1. Albumin as a Molecule for Tissue Engineering Applications

Regenerative medicine seeks to create functionally viable cells in damaged tissue. The culturing of these cells requires a supportive environment that recreates or augments the original milieu. Essential nutrients for metabolism are applied to provide a fluid medium that is tailored to the requirements of the cell type and to the experiment [1]. However, cells live and proliferate better in the presence of biological supplements such as plasma or serum. These provide complex biological molecules including growth factors, hormones, trace elements, attachment and spreading factors, transport molecules, fatty acids, lipids and numerous low molecular weight nutrients [2]. Albumin is the most abundant human plasma protein, accounting for over 50% of the total protein present in the blood stream [3,4], and, as such, is implicated in a variety of roles related to the survival and regeneration of cells. Maurice R. Hilleman and coworkers reported as long ago as 1980 that using fetal bovine serum (FBS) with a complete substitution of human albumin promoted high growth and interferon production by human lymphoblastoid cells [5]. Specifically, bovine fetus serum albumin (BSA) or human serum albumin (HSA) transported in the serum with bound molecules [6–8] seems to account for the positive outcomes. Since then, albumin has been a major component of cell therapy but its growth-

Additional Supporting Information may be found in the online version of this article.

Volume 00, Number 00, Month/Month 2016, Pages 00–00

**Address of correspondence: Denes B. Horvathy, M.D., Institute of Clinical Experimental Research, Semmelweis University, 37-47. Tűzoltó str. Budapest H-1094, Hungary; E-mail: horvathy@yahoo.com*

Received 18 July 2016; accepted 3 October 2016
H.D.B. and S.M. contributed equally in this present work.
DOI 10.1002/biof.1337
Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com)

promoting potential and recently discovered effects raises the need to examine current understanding of it's role in the tissue engineering and regenerative medicine fields.

1.1. Albumin in Cell Culture

Different mammalian cell cultures have been used as models for cell differentiation since the '50s with the aim of maintaining a physiological environment for *in vitro*, organ, or tissue cultures by regulating pH, osmolality, oxygen and carbon dioxide levels. Despite the distinctive requirements of cell types, commercial media are available containing the common salts, sugars, amino acids, and vitamins for an optimal system. Extracts derived from adult or newborn animal tissues, the most widely used of which is FBS, are added to provide biological substrates. Due to donor variability, the concentrations of the components in FBS are not precisely known. However, it is clear that one of its most important proteins is albumin (~23 mg/mL) [9]. The advantages of FBS are (i) its general applicability for cell proliferation and maintenance, since it is a cocktail containing most of the required molecules; (ii) its suitable for both human or animal cells. Independently of the tissue type, FBS is currently the optimal medium supplement for cell culture.

Yet the complexity of FBS-containing cell culture medium introduces variability and uncertainty to experiments, and because its mechanism for facilitating cell growth is not clear, standardizing and increasing the availability of FBS remains problematic. Despite the fact that protocols specify how to extract FBS, small variations in serum content appear among laboratories and from animal to animal. This *batch-to-batch quantitative and qualitative variability* [10] cannot be regulated, and the providers of extracts on the market do not analyze each sample due to time and cost constraints.

To minimize deviations, several methods have been proposed by different laboratories retaining albumin fosters positive alterations in the living conditions of cell culture compared to simple media (Table 1) [11–33]. Since variability in FBS influences cell properties as fundamental as proliferation kinetics and potency, this has wide-scale implications for regulation of cell therapy methodology and FBS supply [34]. Understanding how FBS, including one of its main constituents, albumin, supports cell growth is a crucial step in addressing the challenge of FBS standardization.

To demonstrate this, cell viability was tested on human cancellous bone explants. Bone pieces were cultured under standard conditions with FBS-supplemented Dulbecco's modified Eagle's medium (DMEM). After two days, media were changed and several albumin compositions, including serum albumin and recombinant solutions were alternatively added to DMEM instead of FBS. After 5 days cell number was determined by an XTT cell viability assay. All of the supplemented media resulted in the same cell number, which was slightly increased compared to the unsupplemented fluid (Fig. 1). These results show the beneficial effects of medium supplementation and also show that albumin compositions could be acceptable FBS replacements in terms of simplifying serum additives to cell culture media.

1.2. Albumin and *In Vitro* Fertilization

Autogeneic or allogeneic serum was commonly used in *in vitro* fertilization (IVF) methods as well, however, some patients were found to have embryotoxic factors in the serum [35,36]. Serum heat-inactivation procedures are performed against the transmission of microbes, however these methods do not eliminate prion-mediated diseases like Creutzfeldt-Jacob. At present, the majority of fertility clinics use commercial media in which FBS has been replaced by HSA as a protein source. Aside from the benefit of being purified, HSA seems to have further advantages in IVF outcomes: M. Dhont and coworkers performed a prospective randomized study of patients undergoing IVF or intracytoplasmic sperm injection, where embryos were cultured in Earle's balanced salt solution containing either 8% (v/v) fetal cord serum or 0.4% (w/v) HSA as protein source [37]. Their data demonstrate that the use of the latter supplement is associated with improved morphological embryonic quality and significantly higher pregnancy rates. Furthermore, Bungum compared IVF outcomes by using a standard commercial culture medium containing HSA and a culture medium in which the protein component has been replaced with recombinant human albumin (rHA). They concluded that culture media containing rHA seem to produce embryos of higher quality comparable to media containing HSA, without the risk of contamination [38]. According to these studies, the treatment of cells with autologous, allogeneic or recombinant albumin can provide better results compared to the use of any other supplements.

1.3. Albumin in Transplantation

The addition of albumin may be a useful technique not only for *in vitro* experiments, but also for transplantation. For this, cells or tissues have to be cultured *in vitro* using preservation medium before they are implanted into the human body. Bertera and colleagues observed 30% increase in yields of rodent islets when 0.2% BSA was added to the isolation protocol, and the islet function also improved when BSA was present in the transplantation medium [39]. They suggest that the protease inhibitor activity of BSA suppresses endogenous proteolytic enzyme activity, which could be the reason for higher islet viability. FBS and BSA, because of their xenogenous nature may not be suitable to human cells, so media should be supplemented with an alternative such as human cord blood serum or HSA. For example, the current standard washing protocol for stem cells of cord blood, peripheral blood, and bone marrow is a two-step dilution in the presence of 2.5% HSA [40,41].

1.4. Albumin in Low-Temperature Storage of Cells

Cryopreservation is the maintenance of a variety of tissues, cell types and subcellular materials at sub-zero temperatures for long-term or indefinite storage. To avoid damage to the cell during freezing, a cryoprotective agent (DMSO or glycerol, 10%) is added to the basal medium, and an optimized ratio

TABLE 1 ***Cell culture media***

	Features	*Advantages*	*Disadvantages*	*Albumin content*	*References*
FBS-supplemented media	- Composed of DMEM or α-MEM basal medium, antibiotics and FBS	- All cell type can grow effectively	- *Batch-to-batch* variability and unidentified components	~5 mg/mL	[11–15]
			- Unexpected cell growth characteristics		
			- Risk of possible contamination with viruses, prions, bacteria, mycoplasma, yeast, fungi, and endotoxins		
			- FBS-cultured cells show high immunogenicity in patients		
			- Ethical questions of the blood harvesting of fetal calves		
			- Anti-FBS antibodies were detected after allogeneic transplantation of MSCs cultured in FBS-containing media		
Serum reduced culture media	- The FBS content of the medium is decreased from 10 to 2 v/v % supplemented by known factors in well-defined amount and ratios	- More precisely controlled content of the culture environment	- Unknown factors are still present	Decreased with the dilution factor.	[16]
			- Limited use	Usually ~1 mg/mL	
Chemically defined serum-free media	- Serum free media supplemented with growth factors and nutrients	- Absence of animal compounds (xenofree)	- Has to be characteristic for each cell type, and the long-term effect for the cells has to be examined	Zero or unknown, depending on media type	[17–22]

TABLE 1 *(Continued)*

	Features	*Advantages*	*Disadvantages*	*Albumin content*	*References*
		- Free of microorganisms and prions	- Expensive to produce and test		
		- Safer clinical application possibilities			
Human platelet-derivatives	- Platelets contain large amounts of growth factors and cytokines that are involved in the blood-clotting process	- Can be autologous	- Morphological variances were observed	Platelets endocytose albumin from plasma, the albumin content of their lysate is considerably higher than FBS.	[23–25]
Thrombin-activated platelet releasates (t-PRs)			- Donor-to-donor variations		
Collagen-activated platelet releasates (c-PRs)					
Human platelet lysate (hPL)					
Human serum supplemented medium	Can be autologous or allogeneic	Safe for therapy	Inhibitory effect in case of MSCs	In case of 10% human serum ~5 mg/mL.	[26–29]
			limited availability		
Only-BSA supplemented medium	Major protein of FBS	Ideally doesn't contain unknown molecules	Xenogeneic in case of human cell culture	~5-10 mg/mL	[30,31]
Human serum albumin (HSA)	- Pooled prodcut from healthy donors	About 96% purity	Growth factor content is low	~5-10 mg/mL	[32]
Recombinant human albumin	- Purest source of albumin	- No risk of contamination	- Post-translational modifications are missing	~5–10 mg/mL	[33]
		- No *batch-to-batch* variation	- Less potent activity than serum-derived HSA		

Different albumin supplementation protocols in general cell culture routine.

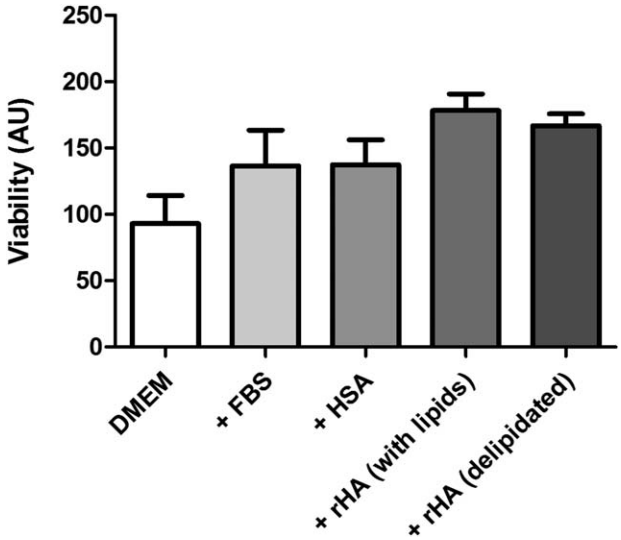

FIG 1 *Cell viability on human cancellous bone explants (XTT assay). FBS supplementation increases cell proliferation as much as HSA and different rHA additives.*

(20-90%) of FBS is added for improved cell viability and cell recovery after thawing. Albumin has shown success as a cryoprotective agent in a variety of specific tissues: Plenert et al. have found that the combination of 20% HSA, 20% autologous serum and 5% DMSO gives the most favourable freezing protection for bone marrow cells [42]. HSA alone was used as the cryoprotective agent in case of human corneal keratocytes [43]. HSA also had a high (more than 25%) cryoprotective effect on the recovery of lyophilized human red blood cells, allowing cellular hemoglobin recovery to exceed 70% [44]. The majority of freezing and thawing solutions used for oocyte vitrification (non-equilibrium freezing) also contain serum albumin [45]. Although in Hreinsson and his coworker's comparison, no significant difference was found between solutions containing serum versus HSA in cryopreservation of follicles [46], reduced viability was seen 4 hr after thawing in culture when cryopreserved with serum, but not HSA [47].

Cryopreservation is the most common way of storing human sperm as well, although it leads to a decline in sperm quality including significant DNA damage [48]. Riel et al. reported a method in which sperm are maintained without freezing in an electrolyte-free medium composed of glucose and bovine serum albumin [49]. Other research groups showed that the additives BSA and FBS provided a protective effect on the motility, morphological integrity, plasma membrane integrity and DNA integrity following liquid storage of sperm up to 72 hr at 5°C [50,51]. They suggest that BSA may provide beneficial factors such as energy substrates or scavenger ions and small molecules.

2. Albumin Binding: More than Just a Carrier?

Approximately 500 g of albumin is present in the human body, mainly as part of the blood circulation, lymphatic system, extra and intercellular compartments. The molecule is a non-glycosylated, helical, heart-shaped polypeptide with a molecular weight of 66,500 Da containing 585 amino acids (Fig. 2). Since the binding effect of albumin was discovered in the 60's [52,53] and the atomic structure was described by

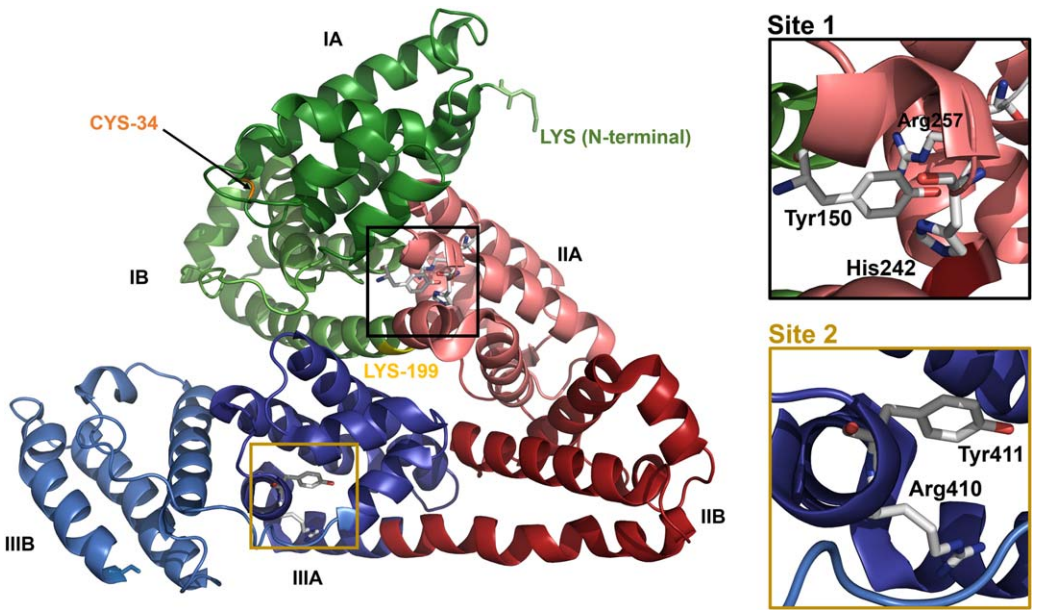

FIG 2 *Schematic drawing of the HSA molecule (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.). Domain IA is shown in green, domain IB in pale green, domain IIA in pink, domain IIB in red, domain IIIA in blue, domain IIIB in pale blue. Details of the two specific drug-binding sites of albumin are shown in inserts.*

TABLE 2 *Metal binding sites*

Binding site	*Metal ion*	*Reference*
N-terminal binding site	Cu^{2+} (picomolar)	[61–63]
	Ni^{2+} (micromolar)	
	Co^{2+}	
Multimetal binding site (MBS A)	Cu^{2+} (micromolar)	[64–66]
	Ni^{2+} (micromolar)	
	Zn^{2+} (micromolar)	
	Cd^{2+} (nanomolar)	
	Mn^{2+}	
Multimetal binding site (MBS B)	Cd^{2+}	[67]
	Co^{2+}	
Sole thiol group of HSA at Cys34	Pt^{2+} and Au^{+} complexes (nanomolar)	[68,69]

Metal binding sites of the albumin molecule.

crystallographic studies conducted in the 90's [54,55], there have been numerous articles examining the characteristics of albumin as a carrier protein. It comprises three binding domains, namely domain I (1-195), II (196-383) and III (384-585). The three domains are further divided into sub-domains A and B [8]. While the domains have similar structures they reveal different ligand-binding affinities and make albumin one of the most promising drug-delivery systems [56]. The main binding sites are shown on Fig. 2 and the ligand binding details are summarized in Tables 2 and 3.

Due to its binding properties, it is an often-revisited hypothesis that the effects exerted by albumin in cell culture media are due at least partly to the molecules it carries rather than albumin *per se*. Although there are no specific studies focusing on this issue alone, it is possible to single out potential effective agents in tissue engineering that are commonly bound to albumin molecules.

2.1. Metal Binding

One prominent binding function of albumin is metal transport, however, specific differences occur between the mammalian albumin types [57–59] and human mutations which may also alter metal ion binding [60]. Typical metal ions transported by HSA are listed in Table 2 [61–69].

Metals transported as complexes may bind several active centers of the HSA molecule. There are experiments concluding that there are ion-specific binding sites, for example, Cd^{2+} binding site [66] and Zn^{2+} binding site [70], which is also a specific Mn^{2+} binding site [67]. Albumin transports other essential metals as well, for example, calcium, iron, mercury, vanadium, but their characterizations are still ongoing [71,72]. Based on the available data, the albumin molecule could be an essential additive in cell culture media because it supplies ions to the system. However, it is unlikely that metal binding plays a significant role in *in vitro* and *in vivo* tissue engineering applications, since necessary ions are generally provided from other sources.

2.2. Fatty Acid Binding

In extracellular fluid, the main role of HSA is the transport of non-esterified fatty acids (FA) [73,74]. HSA works as a detergent, thus enabling the solubilization of fatty acids and ensuring the homogenous distribution through different tissues. Under physiological conditions, 0.1–2 mol fatty acids are bound to 1 mol HSA, but this ratio can rise above 6:1 in case of extreme exercise or under pathological conditions, for example, liver disease, diabetes or cardiovascular diseases [75,76]. Generally, fatty acids with various chain lengths and degree of unsaturation bind to seven binding sites on albumin although eleven fatty acid binding sites have been identified so far [77]. Long-chain fatty acids bind preferentially to the five binding sites initially identified for myristic acid, while medium-chain fatty acids bind preferentially to the drug-binding sites [78].

Fatty acid binding modulates the reactivity of HSA via the –SH group at the Cys34, but, interestingly, FA binding is not at this Cys34 thiol group [79]. This configuration permits synergistic changes in scavenger capacity and antioxidant properties when fatty acids are bound to HSA. Using lipid-rich BSA as an additive of a knockout serum replacement media was

TABLE 3 ***Drug and therapeutic agent binding***

Drug/chemical	*Bond*	*Binding specification*	*Advantage*	*Reference*
Aspirin	Non-covalent association	ε-amino group at low concentration and acetylation of Lys-199 at high concentration	Improved transportation	[55]
Anionic drugs generally	Non-covalent association	IIA	Improved transportation	[8],[82]
Doxorubicin	Non-covalent association	IB	Improved transportation	[83]
Azapropazone, azapropazone-indomethacin, diiodosalicylic acid, iodipamide, oxyphenbutazone, phenylbutazone, phenylbutazone- indomethacin	Myristic acid-HSA complex	site 1 in IIA	Increased half life	[84]
Diflunisal, diazepam, ibuprofen, indoxyl sulfate	HSA-drug complex	site 2 in IIIA	Increased half life	[84]
Levemir	Myristic acid, fatty acid binding	IIA	Improved transportation	[85]
Abraxane	Composite nanoparticle	non-specific binding	Increased water solubility, reduced toxicity	[90]
Technetium^{99m}	Denaturated albumin aggregate	non-specific binding	Improved transportation for imaging	[91]
Nitric oxide	S-nitrosylation	Cys-34	Increased half-life	[92]
INNO 206 (doxorubicin derivative)	Covalent albumin conjugate	Cys-34	High plasma stability, reduced toxicity	[93,94]
AFL-HSA, a fluorescein-labeled albumin conjugate	Covalent albumin conjugate	N-terminal of lysine group	More effective tissue binding for imaging	[95]
Albuferon (fusion protein of albumin and interferon-α-2b (INFα-2b))	Genetic fusion	N-terminal of lysine group	Increased half-life	[96]
Thiol introduced siRNA	Chemical crosslinking (nanosized)	via reactive amine groups converted to thiol groups	Reduced cytotoxicity	[97]
Affibody	secondary bonds	sterically engineered proteins for high affinity	Increased half life	[98]

Drug and therapeutic agent binding of the albumin molecule.

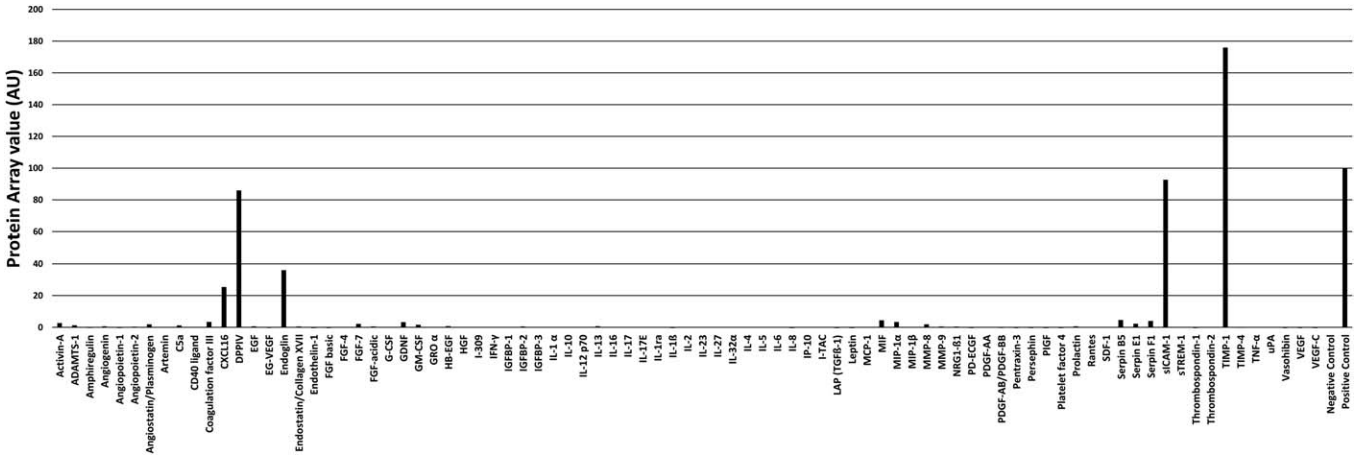

FIG 3

Semiquantitative Proteome Profiler analysis of serum albumin infusion. Using a dot-blot array, 85 different cytokines and growth factors were analyzed. Endoglin, Dipeptidyl peptidase-4, TIMP-1, CXCL16 and siCAM-1 were found in the purified albumin solution, all of which are related to the TGF-β superfamily. Note that other abundant serum-derived proteins, such as VEGF or PDGF are not observed, indicating that those that are there, are so well attached that they survive the industrial albumin purification processes.

also shown to support *in vitro* systems, namely it stimulates long term self-renewal of human embryonic stem cells, it inhibits aggregation in suspension cultures of human induced pluripotent cells and also promotes growth without any influence on pluripotency [80,81]. Fatty acid removal, on the other hand is often included in the purification steps of different albumin solutions, therefore these beneficial effects of the molecule is only active when the molecule becomes lipidated again.

2.3. Drug and Therapeutic Agent Binding

Although the main use of albumin is as a 20% solution blood substitute, a protein present at such concentration with the previously mentioned unique binding properties is also an optimal target for specific drug delivery. Albumin solutions for tissue engineering are prepared from healthy donors who are not on any prescription drugs, so it is unlikely that drug binding interferes with the efficacy of albumin in these settings. Nevertheless, clarifying albumin drug binding is important for understanding its role when applied to tissue engineering. In this section, albumin binding is discussed in relation to drug pharmacokinetics and pharmacodynamics. Specific drug delivery can be achieved by forming albumin complexes, and the main type of complexes used for medical purposes are summarized in Table 3.

According to the listed pharmaceutical formulations, hydrophobic and hydrophilic drugs can be transported bound to albumin throughout the blood circulation system noncovalently at the binding sites on domains IIA, IIIA and IB [8,55,82,83]. Drugs and pharmacological agents containing a fatty acid chain can also bind to albumin at the appropriate fatty acid binding site [84,85]. Fatty acid binding is generally an advantage in the vehicle role of HSA, but may also introduce binding modifications [86]. When using octanoic acid, the HSA binding causes allosteric change at site 2, which resulted in suprofen and ketoprofen displacement. At elevated concentration the acid binds directly at site 1 and can cause drug displacement in a competitive manner [87]. The allosteric effect also took place with the use of oleic-acid isotope [88]. However, it was also proven that competitive binding at the binding sites may also occur using drugs: apomorphine and benserazide binding for example, is inhibited by warfarin [89].

In addition, composite nanoparticles can also be formed to increase drug bioavailability and water solubility and to reduce toxicity [90]. Metal binding sites allow radiopharmaceuticals, for example, Technetium^{99m} derivatives, to be distributed in the bloodstream as suitable tracers for imaging [91]. The well-known vasodilator nitric oxide (NO) forms a covalent bond at Cys34, and also has an increased half-life when bound to albumin [92]. There are more specific products including for example, INNO 206, which is a covalently bound prodrug for higher stability or reduced toxicity [93,94]. Covalent binding can also be formed at the N-terminal of a lysine group to enhance binding to human tissue for better imaging [95]. Another albumin-based delivery system that is a promising formulation is the genetic fusion of INFα-2b with recombinant HA to treat hepatitis C with increased half-life, and thus less frequent dosing [96]. To treat cancer, siRNA and thiolated albumin were crosslinked in a nano-sized formation to reduce toxicity in order to make the treatment more effective [97]. Even the formulation of Affibody contains the drug covalently bound to high affinity HA peptide, proving the transportation and stabilizing effect of albumin [98]. Since albumin improves drug efficiency and controls the release rate of drugs at the target location, HA will likely have an increasingly prominent role in various pharmaceutical fields.

2.4. Cytokine and Growth Factor Binding

Probably the most commonly cited effect of serum albumin in cell culture is that it provides growth factors and cytokines originating from platelets or white blood cells [2]. These factors and endogenous mixtures like platelet rich plasma (PRP) preparations are well investigated in cell culture systems, but no first-hand data is available whether purified albumin preparations contain any growth factors or cytokines. Therefore, we used protein arrays (Human Angiogenesis Array Kit, Cytokine Array Kit, R&D system, #ARY007, #ARY005) to screen for the most suspected growth factors and cytokines, and observed that surprisingly few are detected in a commercially available albumin solution (Serum Albumin, Biotest, 10%, low-salt) (Fig. 3). Interestingly, all that are there are regulated by or themselves regulate the activity of Transforming Growth Factor-β (TGF-β). CXCL16 is an important transmembrane chemokine in the maintenance of bone integrity [99,100]. Endoglin, also known as, CD105, is a component receptor of the TGF-β complex in angiogenesis [101,102]. siCAM-1, the circulating form of ICAM-1 (CD54), is inhibited by TGF-β [103]. As part of the tissue inhibitors of the metalloproteinase family, TIMP-1 has an angiogenic effect in endothelial cells and promotes cell growth in a wide array of cell types, [104]; its recombinant form has been studied for TGF-β regulation in tumour cells [105]. DPPIV, Dipeptidyl peptidase-4, is a protease that promotes cell invasiveness and tumor growth as well as acting as a fibroblast activation protein whose inhibition leads to TGF-β suppression [106,107]. TGF-β has structural and morphological similarities with albumin. TGF-β is a growth factor superfamily that includes a diverse group of cytokines such as bone morphogenetic proteins (BMPs) and activins [108]. These act as cellular and physiologic process regulators in proliferation, differentiation, migration, cell survival, angiogenesis and immunosurveillance [109]. Notably, TGF-β receptors play important roles in albumin binding and endocytosis [108,110]. Siddiqui stated that the endothelial cell surface albumin-binding protein is actually the TGF-β receptor type II, and albumin and TGF-ß are competitive ligands at the TGF-ß receptor [108]. It is important to note that there is a complete omission of expected components, such as PDGF or Platelet factor 4, in the purified albumin preparation screened, indicating that the TGF-β related proteins remain selectively bound to albumin. This observation raises the need for further research to uncover how TGF-ß-related growth factors and cytokines in serum albumin affect its use in tissue engineering.

3. Local Albumin Administration

Even though the intravenous and *in vitro* biotechnological applications of albumin are well-known and the local use of serum products like PRP recieve increasing clinical attention, the local administration of albumin in regenerative processes remains less investigated. It is interesting to consider to what extent albumin's roles are due to its known non-oncotic features like free radical scavenging, neutrophil activation or molecule transportation versus its own biological effects on molecular pathways. In other words, is albumin the active molecule, or is it simply a highly compatible, bacteria repellent scaffold useful for tissue engineering purposes?

3.1. Albumin As a Bacteriostatic Coating of Biomaterials

The anti-bacterial properties of albumin are widely investigated. By raising the negative surface energy and increasing the hydrophility of different biomaterials, albumin functions as an anti-attachment protein and prevents biofilm formation [111,112]. While proteins like fibrinogen, laminin and fibronectin promoted bacterial adherence onto polymethylmethacrylate (PMMA) surfaces, albumin coating almost completely reduced the adherence of *S. aureus* and various coagulase negative *Staphylococcus* strains [113]. In addition, bacterial colonization was also reduced by human albumin coating on polyethylene intravascular catheters *in vitro* [114]. Moreover, Kinnai used HSA to reduce the occlusion and contamination of various tympanoplasty tubes. In these experiments, the authors tested the attachment of fibronectin and showed significantly lower protein amount associated with the albumin coated surface, making the development of reactive granulation tissue less favorable [115]. They have also tested bacterial adherence and found that serum albumin coating significantly reduces *S. aureus* and *P. aeruginosa* contamination on titanium surfaces [116]. Similar results are also available with *S. epidermidis*, showing that cross-linked bovine serum albumin reduces the adherence of the bacteria on titanium surfaces *in vitro* [117,118]. In addition, after intense bacterial exposure, BSA-coated and uncoated implants were inserted in rabbit femora and abscess formation was investigated [119]. Clinical abscess was found in 62% with uncoated material, which was significantly reduced to 27% if BSA-coated implants were used, showing the potential biomedical application in orthopedic interventions. In more recent experiments, Ruiz, del Prado and Naves confirmed that serum albumin applied on polystyrene surface is a potent inhibitor of bacterial (*E. coli, S. pneumoniae*) biofilm formation *in vitro* [112,120,121]. For this reason these authors also suggested the application of albumin coating on biomedical materials like tympanostomy tubes, cochlear implants and urinary catheters in order to reduce complications associated with bacterial colonization.

All implants investigated so far were non-resorbable materials, but in tissue engineering, the implants are expected to reabsorb and remodel into living tissue. Albumin coating as a potential anti-bacterial agent has not been investigated so far on any resorbable materials, though it has widespread applications in clinical settings like in routine traumatologic injuries or septic implant revisions. This research line therefore has further implications for sutures, cartilage scaffolds, or even living tissues.

3.2. Albumin as an Attachment Protein for Cells

In contrast to the anti-attachment characteristics with bacteria, albumin was shown to promote the adherence of eukaryotic cells. Different biomaterials with albumin coating are under investigation. We used serum albumin as an attachment protein for stem cells on absorbable suture materials, whereby the protein coating was achieved through a freeze-drying step [122]. We found significantly higher attached cell number 48 hr after the seeding on the albumin coated surface compared to fibronectin or poly-l-lysine. Bioactive sutures were then implanted into rat skeletal muscle, where stem cells detached from the vehicle and migrated deeper into the tissue, proving the method to be successful for cell delivery. This technique could be useful in surgical interventions, allowing tissue repair with sutures despite the presence of only a limited number of multipotent cells.

Bone-related biomaterials coated with albumin are also studied as adherence facilitators for various cell types. Bernard investigated cell attachment onto hydroxyapatite (HAP) coated with bone sialoprotein and osteopontin [123]. BSA was used to block non-specific binding sites, but interestingly, BSA attached the highest number of osteoblasts. Importantly, they have also tested other commercially available albumin solutions and found the same attachment pattern, which suggests that albumin itself, and not impurity of the solution, promotes cell adhesion. In these experiments, the albumin coating was achieved by soaking the material in heat-denatured solution and the adsorbed albumin influenced osteoblast attachment in a concentration-dependent manner. They concluded that even though albumin does not have a known cell binding site, it influences cell adhesion and proliferation positively, therefore albumin should be incorporated into biomaterials to improve bone regeneration [123].

We found similar results with lyophilized bone allografts [124]. In this experiment mesenchymal stem cell adherence was tested at 3 and 18 days after seeding. Albumin attracted mesenchymal stem cells significantly better compared to fibronectin and collagen 1, an effect that was further enhanced under dynamic seeding conditions [124]. According to this study, the albumin-coated surface not only increased the attachment of stem cells, but also supported their long-term survival and proliferation. The phenomenon, however, was only present if freeze-drying was used to attach the albumin to the allograft surface. This supports the concentration-dependent characteristics of albumin described by Bernards, since the adsorbed amount of serum albumin was potentially higher after freeze-drying compared to soaking [123]. Additionally, these albumin-coated mineralized allografts successfully bridged non-union femoral defects and increased trabecular thickness in rats, while uncoated materials failed [124,125]. This animal study has recently been confirmed in humans. First as a case series, then as a double-blind controlled study we shown that serum albuin coated bone allografts show excellent remodeling characteristics in patients

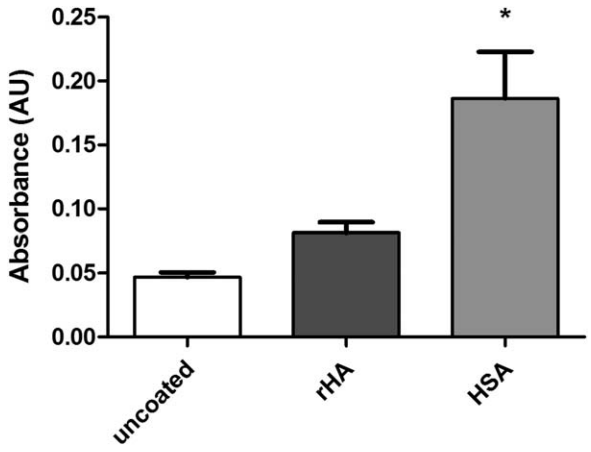

FIG 4 *Cellular attachment pattern after 12 hr on demineralized bone matrix with rHA and HSA coatings. MTT cell viability test showed that rHA slightly increases cell attachment, while serum albumin coating resulted in significantly higher cell numbers (One-Way ANOVA,* $P < 0.001$*).*

[126,127]. Another widely used bone substitute was also tested with albumin coating. Our recent data showed the same attachment pattern of mesenchymal stem cells on albumin coated demineralized bone matrix (DBM) after 6, 12 and 24 hr [128]. DBM alone is capable of fully regenerating even bone defects of critical size, but due to the low concentration of osteogenic factors, the process is rather slow [129]. By making the biomaterial attractive for multipotent cells, albumin possibly increases the local stem cell number and speeds up the ossification. In fact, after implanting albumin-coated DBM into rat critical size calvaria defects, faster ossification, increased bone volume and mechanically stronger bone proved the efficacy of the albumin treatment [128]. In addition, Liu investigated the surface modifications of BSA-treated titanium nanotubes and tested the early attachment, spreading and proliferation of human gingival fibroblasts [130]. According to this study, surface roughness plays an important role in cellular attachment, and by coating the inert biomaterial with BSA, the surface irregularity is increased, creating a much more favorable environment for fibroblast adherence. In addition, albumin raised the negative surface energy and increased the hydrophilicity, which promotes macromolecule adsorption eventually leading towards eukaryotic cell adherence [130]. Interestingly, the same mechanisms and characteristics are responsible for making the surface repellent for bacteria. Liu also highlighted that by being suitable for gingival fibroblasts and unattractive for bacteria, albumin coating potentionally improves the integration of titanium implants and simultaneously reduces infectious complications in oral cavity interventions [130]. In the above mentioned experiments albumin was used as a purified serum fraction, which raises the question whether albumin alone or the attached cytokines and growth factors are responsible for the attachement pattern. To investigate this issue, we completed an adherence study with rat

mesenchymal stem cells and DBMs using recombinant albumin as well. We found that after 12 hr of incubation the recombinant protein slightly increased cell attachment, while the serum originated albumin resulted in even higher cell number (Fig. 4). According to these results, albumin by itself is a potent attachment protein, but accessory molecules in the albumin solutions could have an important role in making the surface more suitable for cellular adherence. We showed that the recombinant and the serum originated protein similarly increased cell proliferation on bone explants (Fig. 1). As a medium additive, however, the recombinant protein could immediately function as a carrier and attach molecules produced by the cultured cells. In the attachment experiment on the other hand the time was rather limited for this action, since unattached cells are less metabolically active. Taken together, these data indicate that albumin coating on implants, either resorbable or permanent, favors human cell colonization and blocks bacterial contamination, as a result supporting a more successful implantation.

3.3. Albumin Scaffolds

Engineered tissues grow on scaffolds that support their growth and guide regeneration. A new approach for the production of albumin scaffolds is based on the electrospinning of albumin fibres [131,132]. This requires a fibre-forming agent, for example, beta-mercaptoethanol [131] or polyethylene oxide [132], since the native form of albumin is globular. The scaffolds can either have a mat-like form [132], or be tailored to a vessel-like morphology [131]. Cross-linking of HSA can be achieved using microbial transglutaminase or glutaraldehyde as polymerizing agents [133–135], Li showed that albumin scaffolds produced mechanical and physical results *in vitro* similar to those of collagen-based scaffolds in osteogenic induction medium. Whereas collagen-based, natural scaffolds are limited by their rapid degradation, the albumin tissue scaffolds maintained their dimension and shape 28 days later [135]. Biocompatibility and biodegradability are considerations for *in vitro* and *in vivo* use of electrospun albumin scaffolds. Nseir concludes that albumin scaffolds are biodegradable and provoke a milder inflammatory response compared to synthetic scaffolds, which were furthermore not enzymatically degraded as the albumin scaffolds were [131]. Noszczyk found that the albumin electrospun mats can be used as antiadhesive dressing *in vitro* due to their cell suppression characteristics; *in vivo*, the mats are completely resorbable in 6 days and elicit only limited local inflammatory responses [132].

Gallego performed both *in vivo* and *in vitro* studies to verify the biocompatibility of the scaffold, and found induction of ectopic bone formation and no inflammatory response at the scaffold surface [134]. Human osteoblasts are capable of closing critical-sized rat mandibular defects in conjunction with the scaffold [36]. Another approach is the incorporation of albumin into ceramic scaffolds. Mueller suggests the incorporation of albumin into complex-shaped HAP scaffolds as biodegradable bone substitutes or drug delivery platforms [136]. These scaffolds can be used in non-load bearing applications. After 8 weeks of implantation, the hydroxyapatite (HA) scaffold with albumin *in vivo* had the highest bone formation and bone regeneration rates [136]. Hess suggests that it is possible to obtain HA/protein scaffolds with controllable properties, concluding that adding BSA can tailor the porosity and adding fibrinogen increases the material's strength. This illustrates how active proteins can be directly incorporated into a ceramic suspension by sample preparation [136]. Together, experiments point to the use of albumin, either alone or in combination with traditional materials, as promising scaffolds in tissue engineering applications.

3.4. Albumin As the Active Agent in Bone Regeneration

There are already a few studies in which albumin proves to be an acting molecule, not only a bacteriostatic structure, biomaterial coating or highly biocompatible scaffold. According to *in vitro* studies, serum albumin probably recruits endogenous stem cells, and thereby fuels bone regeneration with potential progenitors locally [124,125,128]. Once cells are present, albumin also provides a convenient millieu for osteoblast function according to previously described findings from Gallego [134]. The idea also supports the *in vivo* results from Klara, who demonstrated intensive osteoblast function twelve months after implanting large structural allografts with albumin in revision arthroplasty [126]. Even though there are experiments investigating albumin as the active molecule, the mode of action is not well elucidated. An important series of study was completed by Yamaguchi et al. investigating albumin in bone regeneration experiments. First, they have showed that albumin production increases locally after bone fracture [137]. They also tested bovine serum albumin as a medium supplementation on bone explants *in vitro* and showed that albumin addition increases the calcium and DNA content of the bone fragments [137]. Next, they have showed that not only hepatic cells are capable of albumin production, but bone marrow derived cells and osteoblasts also have this capability [138]. Moreover, osteoblasts have receptors for bone stimulating molecules like PTH and IGF-1. Interestingly, albumin production can also be enhanced by these molecules, proving again the connection between osteoblast function and albumin production [138]. Further investigation revealed increased proliferation of osteoblasts after albumin supplementation in the cell culture media, possibly responsible for the growing DNA content in bone fragments as well [139].

Albumin supplementation also decreased the ALP activity of osteoblasts via inhibition of the Runx2 pathway. The suppression of the Runx2 pathway inhibits the differentiation of the osteoblasts, therefore albumin probably indirectly supports the proliferation activity [140]. Since the authors showed abolished effects of albumin supplementation after inhibiting molecular pathways, they suggested that albumin behaves as an extracellular signaling molecule, but the receptor and the precise pathway are yet unknown [141]. The existence of a

specific albumin receptor was already mentioned in 1981, but no evidence was found since then [142]. The theory is recently supported by the fact that a very similar molecule, AFP does have a specific receptor [142] and that the albumin molecule uses receptor mediated endocytosis to deliver its ligands to the intracellular compartment [142–144]. Moreover, the albumin molecule has a relatively long half-life in serum, which is due to the salvaging mechanisms of albumin-binding cellular receptors [145,146]. The neonatal Fc receptor (FcRn), for example, protects the albumin molecule from intracellular degradation, which provides equivalent amounts of albumin as the liver produces [147], while the cubulin-megalin complex supports reabsorbtion from the glomerular filtrate [146]. Other surface glycoproteins, like gp18, 30 and 60 also have albumin binding affinity and play an important role in the homeostasis of the molecule [145,146]. Even though these albumin binding receptors exist, no certain pathway was identified proving the albumin molecule to be an extracelluar messenger.

Even so, albumin seems to act as a self-supporting molecule after bone injury, since it is produced by osteoblasts at the trauma site and increases cell proliferation locally. According to the *in vitro* findings showing increased stem cell adherence onto albumin coated surfaces, it is also possible that the therapeutically increased local concentration of albumin recruits endogenous osteoblasts and supports their proliferation. After the albumin concentration decreases, osteoblasts differentiate resulting in faster ossification. This mode of action supports the *in vivo* experiments where serum albumin coated allografts and DBM were shown to successfully treat various bone defects [124–126,128]. Thus, albumin possibly supports bone tissue regeneration as an active molecule in addition to its bacteriostatic behavior and cell attachment properties.

4. Conclusion

Various serum fractions such as platelet-rich plasma have been at the forefront of current tissue engineering approaches. Serum albumin, a major plasma protein, is ubiquitous and complex in ways that are still being uncovered. By elucidating mechanisms of the albumin molecule's multiple roles as binding protein, carrier, scaffold, and active agent, we can harness its potential for functional healing and tissue remodeling. It acts as a pro-attachment protein for mammalian cells, especially stem cells, while at the same time blocking bacterial colonization, making it a very favorable coating material for tissue engineering implants. Moreover, albumin acts as a carrier molecule for cytokines and growth factors. In addition, serum albumin shows direct beneficial regenerative effects in tissue engineering applications, promisingly in bone healing. These novel treatment strategies will benefit greatly from investigating albumin and redefining its place in the field of tissue engineering and regenerative medicine.

Conflict of Interest

I.H. and Z.L. hold a granted patent on serum albumin coated bone allografts and are stakeholders in OrthoSera GmbH, a startup established to develop the technology towards clinical use.

References

[1] Butler, M., and Jenkins, H. (1989) Nutritional aspects of the growth of animal cells in culture. J. Biotechnol. 12, 97–110.

[2] Francis, G. L. (2009) Albumin and mammalian cell culture: implications for biotechnology applications. Cytotechnology 62, 1–16.

[3] Elsadek, B., and Kratz, F. (2012) Impact of albumin on drug delivery–new applications on the horizon. J. Control Release 157, 4–28.

[4] Peters, T. Jr, and Stewart, A. J. (2013) Albumin research in the 21st century. Biochim. Biophys. Acta. 1830, 5351–5353.

[5] Field, A. K., Scattergood, E. M., Hopke, C. J., Tytell, A. A., and Hilleman, M. R. (1980) Replacement of animal serum proteins by human albumin for growth and interferon production by Namalva cells. Antimicrob. Agent. Chemother. 18, 930.

[6] Bertucci, C., and Domenici, E. (2002) Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. Curr. Med. Chem. 9, 1463–1481.

[7] Spector, A.A., John, K., and Fletcher, J. E. (1969) Binding of long-chain fatty acids to bovine serum albumin. J. Lipid Res. 10, 56–67.

[8] Yang, F., Zhang, Y., and Liang, H. Interactive association of drugs binding to human serum albumin. Int. J. Mol. Sci. 15, 3580–3595.

[9] Price, P., and Gregory, E. (1982) Relationship between *in vitro* growth promotion and biophysical and biochemical properties of the serum supplement. In Vitro 18, 576–584.

[10] Tekkatte, C., Gunasingh, G. P., Cherian, K.M., and Sankaranarayanan, K. (2011) "Humanized" stem cell culture techniques: the animal serum controversy. Stem Cells Int. 2011, 504723. doi: 10.4061/2011/504723.

[11] Jochems, C. E., van der Valk, J. B., Stafleu, F. R., and Baumans, V. (2002) The use of fetal bovine serum: ethical or scientific problem?. Altern. Lab. Anim. 30, 219–227.

[12] Selvaggi, T. A., Walker, R. E., and Fleisher, T. A. (1997) Development of antibodies to fetal calf serum with arthus-like reactions in human immunodeficiency virus-infected patients given syngeneic lymphocyte infusions. Blood. 89, 776–779.

[13] Erickson, G. A., Bolin, S. R., and Landgraf, J. G. (1991) Viral contamination of fetal bovine serum used for tissue culture: risks and concerns. Dev. Biol. Stand. 75, 173–175.

[14] Horwitz, E. M., Gordon, P. L., Koo, W. K., Marx, J. C., Neel, M. D., McNall, R. Y., Muul, L., and Hofmann, T. (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc. Natl. Acad. Sci. U S A. 99, 8932–8937.

[15] Sundin, M., Ringden, O., Sundberg, B., Nava, S., Gotherstrom, C., and Le Blanc, K. (2007) No alloantibodies against mesenchymal stromal cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients. Haematologica 92, 1208–1215.

[16] Wappler, J., Rath, B., Laufer, T., Heidenreich, A., and Montzka, K. (2013) Eliminating the need of serum testing using low serum culture conditions for human bone marrow-derived mesenchymal stromal cell expansion. Biomed. Eng. Online 12, 15.

[17] Brunner, D., Frank, J., Appl, H., Schoffl, H., Pfaller, W., and Gstraunthaler, G. (2010) Serum-free cell culture: the serum-free media interactive online database. Altex 27, 53–62.

[18] Chase, L. G., Lakshmipathy, U., Solchaga, L. A., Rao, M. S., and Vemuri, M. C. (2010) A novel serum-free medium for the expansion of human mesenchymal stem cells. Stem Cell. Res. Ther. 1, 8.

[19] Barnes, D., and Sato, G. (1980) Serum-free cell culture: a unifying approach. Cell 22, 649–655.

[20] van der Valk, J., Brunner, D., De Smet, K., Fex Svenningsen, A., Honegger, P., Knudsen, L. E., Lindl, T., Noraberg, J., Price, A., Scarino, M. L., and Gstraunthaler, G. (2010) Optimization of chemically defined cell culture media–replacing fetal bovine serum in mammalian *in vitro* methods. Toxicol. in Vitro 24, 1053–1063.
[21] van der Valk, J., Mellor, D., Brands, R., Fischer, R., Gruber, F., Gstraunthaler, G., Hellebrekers, L., Hyllner, J., Jonker, F. H., Prieto, P., Thalen, M., and Baumans, V. (2004) The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. Toxicol. in Vitro 18, 1–12.
[22] Bjare, U. (1992) Serum-free cell culture. Pharmacol. Ther. 53, 355–374.
[23] Doucet, C., Ernou, I., Zhang, Y., Llense, J. R., Begot, L., Holy, X., and Lataillade, J. J. (2005) Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. J. Cell Physiol. 205, 228–236.
[24] Johansson, L., Klinth, J., Holmqvist, O., and Ohlson, S. (2003) Platelet lysate: a replacement for fetal bovine serum in animal cell culture? Cytotechnology 42, 67–74.
[25] Kramer, D. K., Bouzakri, K., Holmqvist, O., Al-Khalili, L., and Krook, A. (2005) Effect of serum replacement with plysate on cell growth and metabolismin primary cultures of human skeletal muscle. Cytotechnology 48, 89–95.
[26] Shahdadfar, A., Fronsdal, K., Haug, T., Reinholt, F. P., and Brinchmann, J. E. (2005) In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cell. 23, 1357–1366.
[27] Berger, M. G., Veyrat-Masson, R., Rapatel, C., Descamps, S., Chassagne, J., and Boiret-Dupre, N. (2006) Cell culture medium composition and translational adult bone marrow-derived stem cell research. Stem Cell. 24, 2888–2890.
[28] Stute, N., Holtz, K., Bubenheim, M., Lange, C., Blake, F., and Zander, A. R. (2004) Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. Exp. Hematol. 32, 1212–1225.
[29] Chachques, J. C., Herreros, J., Trainini, J., Juffe, A., Rendal, E., Prosper, F., and Genovese, J. (2004) Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. Int. J. Cardiol. 95 Suppl 1, S29–S33.
[30] Nilausen, K. (1978) Role of fatty acids in growth-promoting effect of serum albumin on hamster cells in vitro. J. Cell. Physiol. 96, 1–14.
[31] Fitzsimmons, J. S., Sanyal, A., Gonzalez, C., Fukumoto, T., Clemens, V. R., O'Driscoll, S. W., and Reinholz, G. G. (2004) Serum-free media for periosteal chondrogenesis in vitro. J. Orthop. Res. 22, 716–725.
[32] Barbaro, B., Kuechle, J., Salehi, P., Rodriguez, L., Qi, M., Gangemi, A., Benedetti, E., and Oberholzer, J. (2008) Increased albumin concentration reduces apoptosis and improves functionality of human islets. Artif. Cell. Blood Substit. Immobil. Biotechnol. 36, 74–81.
[33] Keenan, J., Pearson, D., and Clynes, M. (2006) The role of recombinant proteins in the development of serum-free media. Cytotechnology 50, 49–56.
[34] Brindley, D. A., Davie, N. L., Culme-Seymour, E. J., Mason, C., Smith, D. W., and Rowley, J. A. (2012) Peak serum: implications of serum supply for cell therapy manufacturing. Regen. Med. 7, 7–13.
[35] Leveille, M. C., Carnegie, J., and Tanphaichitr, N. (1992) Effects of human sera and human serum albumin on mouse embryo culture. J. Assist. Reprod. Genet. 9, 45–52.
[36] Dokras, A., Sargent, I. L., Redman, C. W., and Barlow, D. H. (1993) Sera from women with unexplained infertility inhibit both mouse and human embryo growth in vitro. Fertil. Steril. 60, 285–292.
[37] Laverge, H., De Sutter, P., Desmet, R., Van der Elst, J., and Dhont, M. (1997) Prospective randomized study comparing human serum albumin with fetal cord serum as protein supplement in culture medium for in-vitro fertilization. Hum. Reprod. (Oxford, England) 12, 2263–2266.
[38] Bungum, M., Humaidan, P., and Bungum, L. (2002) Recombinant human albumin as protein source in culture media used for IVF: a prospective randomized study. Reprod. Biomed. Online 4, 233–236.
[39] Bertera, S., Balamurugan, A. N., Bottino, R., He, J., and Trucco, M. (2012) Increased yield and improved transplantation outcome of mouse islets with bovine serum albumin. J. Transplant. 2012, 856386.
[40] Cilloni, D., Garau, D., Regazzi, E., Sammarelli, G., Savoldo, B., Caramatti, C., Mangoni, L., Rizzoli, V., and Carlo-Stella, C. (1999) Primitive hematopoietic progenitors within mobilized blood are spared by uncontrolled rate freezing. Bone Marrow Transplant 23, 497–503.
[41] Berz, D., McCormack, E. M., Winer, E. S., Colvin, G. A., and Quesenberry, P. J. (2007) Cryopreservation of hematopoietic stem cells. Am. J. Hematol. 82, 463–472.
[42] Reiners, B., Zintl, F., and Plenert, W. (1987) [Use of human albumin as an additional cryoprotective agent in freeze preservation of hematopoietic stem cells]. Folia Haematol. Int. Mag. Klin. Morphol. Blutforsch 114, 264–272.
[43] Borderie, V. M., Lopez, M., Lombet, A., Carvajal-Gonzalez, S., Cywiner, C., and Laroche, L. (1998) Cryopreservation and culture of human corneal keratocytes. Invest. Ophthalmol. Vis. Sci. 39, 1511–1519.
[44] Han, Y., Quan, G. B., Liu, X. Z., Ma, E. P., Liu, A., Jin, P., and Cao, W. (2005) Improved preservation of human red blood cells by lyophilization. Cryobiology 51, 152–164.
[45] Elnahas, A., Alcolak, E., Marar, E. A., Elnahas, T., Elnahas, K., Palapelas, V., Diedrich, K., and Al-Hasani, S. (2010) Vitrification of human oocytes and different development stages of embryos: An overview. Middle East Fertil. Soc. J. 15, 2–9.
[46] Hreinsson, J., Zhang, P., Swahn, M. L., Hultenby, K., and Hovatta, O. (2003) Cryopreservation of follicles in human ovarian cortical tissue. Comparison of serum and human serum albumin in the cryoprotectant solutions. Hum. Reprod. (Oxford, England) 18, 2420–2428.
[47] Hovatta, O. (2005) Methods for cryopreservation of human ovarian tissue. Reprod. BioMed. Online 10, 729–734.
[48] Said, T. M., Gaglani, A., and Agarwal, A. (2010) Implication of apoptosis in sperm cryoinjury. Reprod. BioMed. Online 21, 456–462.
[49] Riel, J. M., Yamauchi, Y., Huang, T. T., Grove, J., and Ward, M. A. (2011) Short-term storage of human spermatozoa in electrolyte-free medium without freezing maintains sperm chromatin integrity better than cryopreservation. Biol. Reprod. 85, 536–547.
[50] Sarıözkan, S., Türk, G., Cantürk, F., Yay, A., Eken, A., and Akçay, A. (2013) The effect of bovine serum albumin and fetal calf serum on sperm quality, DNA fragmentation and lipid peroxidation of the liquid stored rabbit semen. Cryobiology 67, 1–6.
[51] De Leeuw, F. E., De Leeuw, A. M., Den Daas, J. H. G., Colenbrander, B., and Verkleij, A. J. (1993) Effects of various cryoprotective agents and membrane-stabilizing compounds on bull sperm membrane integrity after cooling and freezing. Cryobiology 30, 32–44.
[52] Peters, T. Jr., and Blumenstock, F. A. (1967) Copper-binding properties of bovine serum albumin and its amino-terminal peptide fragment. J. Biol. Chem. 242, 1574–1578.
[53] Peters, T. Jr., and Hawn, C. (1967) Isolation of two large peptide fragments from the amino- and carboxyl-terminal positions of bovine serum albumin. J. Biol. Chem. 242, 1566–1573.
[54] Carter, D. C., Chang, B., Ho, J. X., Keeling, K., and Krishnasami, Z. (1994) Preliminary crystallographic studies of four crystal forms of serum albumin. Eur. J. Biochem. 226, 1049–1052.
[55] He, X. M., and Carter, D. C. (1992) Atomic structure and chemistry of human serum albumin. Nature 358, 209–215.
[56] Kratz, F., and Elsadek, B. (2012) Clinical impact of serum proteins on drug delivery. J. Control Release 161, 429–445.
[57] Appleton, D. W., and Sarkar, B. (1971) The absence of specific copper (II)-binding site in dog albumin. A comparative study of human and dog albumins. J. Biol. Chem. 246, 5040–5046.
[58] Callan, W. M., and Sunderman, F. W. Jr. (1973) Species variations in binding of 63 NI(II) by serum albumin. Res. Commun. Chem. Pathol. Pharmacol. 5, 459–472.
[59] Predki, P. F., Harford, C., Brar, P., and Sarkar, B. (1992) Further characterization of the N-terminal copper(II)- and nickel(II)-binding motif of proteins. Studies of metal binding to chicken serum albumin and the native sequence peptide. Biochem. J. 287 (Pt 1), 211–215.
[60] Kragh-Hansen, U., Brennan, S. O., Minchiotti, L., and Galliano, M. (1994) Modified high-affinity binding of Ni2+, Ca2+ and Zn2+ to natural mutants

of human serum albumin and proalbumin. Biochem. J. 301 (Pt 1), 217–223.
[61] Glennon, J. D., and Sarkar, B. (1982) Nickel(II) transport in human blood serum. Studies of nickel(II) binding to human albumin and to native-sequence peptide, and ternary-complex formation with L-histidine. Biochem. J. 203, 15–23.
[62] Laussac, J. P., and Sarkar, B. (1984) Characterization of the copper(II)- and nickel(II)-transport site of human serum albumin. Studies of copper(II) and nickel(II) binding to peptide 1-24 of human serum albumin by 13C and 1H NMR spectroscopy. Biochemistry 23, 2832–2838.
[63] Rozga, M., Sokolowska, M., Protas, A. M., and Bal, W. (2007) Human serum albumin coordinates Cu(II) at its N-terminal binding site with 1 pM affinity. J. Biol. Inorg. Chem. 12, 913–918.
[64] Bal, W., Christodoulou, J., Sadler, P. J., and Tucker, A. (1998) Multi-metal binding site of serum albumin. J. Inorg. Biochem. 70, 33–39.
[65] Sadler, P. J., and Viles, J. H. (1996) 1H and (113)Cd NMR investigations of Cd(2+) and Zn(2+) binding sites on serum albumin: competition with Ca(2+), Ni(2+), Cu(2+), and Zn(2+). Inorg. Chem. 35, 4490–4496.
[66] Fanali, G., Cao, Y., Ascenzi, P., and Fasano, M. (2012) Mn(II) binding to human serum albumin: a (1)H-NMR relaxometric study. J. Inorg. Biochem. 117, 198–203.
[67] Mothes, E., and Faller, P. (2007) Evidence that the principal CoII-binding site in human serum albumin is not at the N-terminus: implication on the albumin cobalt binding test for detecting myocardial ischemia. Biochemistry 46, 2267–2274.
[68] Gonias, S. L., and Pizzo, S. V. (1983) Complexes of serum albumin and cis-dichlorodiammineplatinum (II). The role of cysteine 34 as a nucleophilic entering group and evidence for reaction between bound platinum and a second macromolecule. J. Biol. Chem. 258, 5764–5769.
[69] Christodoulou, J., Sadler, P. J., and Tucker, A. (1995) 1H NMR of albumin in human blood plasma: drug binding and redox reactions at Cys34. FEBS Lett. 376, 1–5.
[70] Goumakos, W., Laussac, J. P., and Sarkar, B. (1991) Binding of cadmium(II) and zinc(II) to human and dog serum albumins. An equilibrium dialysis and 113Cd-NMR study. Biochem. Cell. Biol. 69, 809–820.
[71] Bal, W., Sokolowska, M., Kurowska, E., and Faller, P. (2013) Binding of transition metal ions to albumin: sites, affinities and rates. Biochim. Biophys. Acta 1830, 5444–5455.
[72] Sokolowska, M., Wszelaka-Rylik, M., Poznanski, J., and Bal, W. (2009) Spectroscopic and thermodynamic determination of three distinct binding sites for Co(II) ions in human serum albumin. J. Inorg. Biochem. 103, 1005–1013.
[73] Cistola, D. P. (1998) Fat sites found! Nat. Struct. Biol. 5, 751–753.
[74] Carter, D. C., and Ho, J. X. (1994) Structure of serum albumin. Adv. Protein Chem. 45, 153–203.
[75] Brodersen, R., Andersen, S., Vorum, H., Nielsen, S. U., and Pedersen, A. O. (1990) Multiple fatty acid binding to albumin in human blood plasma. Eur. J. Biochem. 189, 343–349.
[76] Cistola, D. P., and Small, D. M. (1991) Fatty acid distribution in systems modeling the normal and diabetic human circulation. A 13C nuclear magnetic resonance study. J. Clin. Invest. 87, 1431–1441.
[77] Bhattacharya, A. A., Grune, T., and Curry, S. (2000) Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. J. Mol. Biol. 303, 721–732.
[78] Colmenarejo, G. (2003) In silico prediction of drug-binding strengths to human serum albumin. Med. Res. Rev. 23, 275–301.
[79] Pavicevic, I. D., Jovanovic, V. B., Takic, M. M., Penezic, A. Z., Acimovic, J. M., and Mandic, L. M. (2014) Fatty acids binding to human serum albumin: changes of reactivity and glycation level of Cysteine-34 free thiol group with methylglyoxal. Chem. Biol. Interact. 224C, 42–50.
[80] Garcia-Gonzalo, F. R., and Izpisua Belmonte, J. C. (2008) Albumin-associated lipids regulate human embryonic stem cell self-renewal. PLoS One, 3, e1384.
[81] Horiguchi, I., and Sakai, Y. (2016) Serum replacement with albumin-associated lipids prevents excess aggregation and enhances growth of induced pluripotent stem cells in suspension culture. Biotechnol. Prog. 32, 1009–1016.
[82] Schonfeld, D. L., Ravelli, R. B., Mueller, U., and Skerra, A. (2008) The 1.8-A crystal structure of alpha1-acid glycoprotein (Orosomucoid) solved by UV RIP reveals the broad drug-binding activity of this human plasma lipocalin. J. Mol. Biol. 384, 393–405.
[83] Zsila, F. (2013) Subdomain IB is the third major drug binding region of human serum albumin: toward the three-sites model. Mol. Pharm., 10, 1668–1682.
[84] Ghuman, J., Zunszain, P. A., Petitpas, I., Bhattacharya, A. A., Otagiri, M., and Curry, S. (2005) Structural basis of the drug-binding specificity of human serum albumin. J. Mol. Biol. 353, 38–52.
[85] Morales, J. (2007) Defining the role of insulin detemir in Basal insulin therapy. Drugs 67, 2557–2584.
[86] Menke, G., Worner, W., Kratzer, W., and Rietbrock, N. (1989) Kinetics of drug binding to human serum albumin: allosteric and competitive inhibition at the benzodiazepine binding site by free fatty acids of various chain lengths. Naunyn Schmiedebergs Arch. Pharmacol. 339, 42–47.
[87] Noctor, T. A., Wainer, I. W., and Hage, D. S. (1992) Allosteric and competitive displacement of drugs from human serum albumin by octanoic acid, as revealed by high-performance liquid affinity chromatography, on a human serum albumin-based stationary phase. J. Chromatogr. 577, 305–315.
[88] Jafari, N., Ahmed, R., Gloyd, M., Bloomfield, J., Britz-McKibbin, P., and Melacini, G. (2016) Allosteric sensing of fatty acid binding by NMR: application to human serum albumin. J. Med. Chem. 59, 7457–7465.
[89] Fanali, G., Rampoldi, V., di Masi, A., Bolli, A., Lopiano, L., Ascenzi, P., and Fasano, M. (2010) Binding of anti-Parkinson's disease drugs to human serum albumin is allosterically modulated. IUBMB Life 62, 371–376.
[90] Gradishar, W. J., Tjulandin, S., Davidson, N., Shaw, H., Desai, N., Bhar, P., Hawkins, M., and O'Shaughnessy, J. (2005) Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J. Clin. Oncol. 23, 7794–7803.
[91] van der Poel, H. G., Buckle, T., Brouwer, O. R., Valdes Olmos, R. A., and van Leeuwen, F. W. (2011) Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. Eur. Urol. 60, 826–833.
[92] Ishima, Y., Sawa, T., Kragh-Hansen, U., Miyamoto, Y., Matsushita, S., Akaike, T., and Otagiri, M. (2007) S-Nitrosylation of human variant albumin Liprizzi (R410C) confers potent antibacterial and cytoprotective properties. J. Pharmacol. Exp. Ther. 320, 969–977.
[93] Kratz, F., Warnecke, A., Scheuermann, K., Stockmar, C., Schwab, J., Lazar, P., Druckes, P., Esser, N., Drevs, J., Rognan, D., Bissantz, C., Hinderling, C., Folkers, G., Fichtner, I., and Unger, C. (2002) Probing the cysteine-34 position of endogenous serum albumin with thiol-binding doxorubicin derivatives. Improved efficacy of an acid-sensitive doxorubicin derivative with specific albumin-binding properties compared to that of the parent compound. J. Med. Chem. 45, 5523–5533.
[94] Graeser, R., Esser, N., Unger, H., Fichtner, I., Zhu, A., Unger, C., and Kratz, F. (2010) INNO-206, the (6-maleimidocaproyl hydrazone derivative of doxorubicin), shows superior antitumor efficacy compared to doxorubicin in different tumor xenograft models and in an orthotopic pancreas carcinoma model. Invest. New Drug. 28, 14–19.
[95] Kremer, P., Wunder, A., Sinn, H., Haase, T., Rheinwald, M., Zillmann, U., Albert, F. K., and Kunze, S. (2000) Laser-induced fluorescence detection of malignant gliomas using fluorescein-labeled serum albumin: experimental and preliminary clinical results. Neurol. Res. 22, 481–489.
[96] Flisiak, R., and Flisiak, I. (2010) Albinterferon-alpha 2b: a new treatment option for hepatitis C. Exp. Opin. Biol. Ther. 10, 1509–1515.
[97] Son, S., Song, S., Lee, S. J., Min, S., Kim, S. A., Yhee, J. Y., Huh, M. S., Chan Kwon, I., Jeong, S. Y., Byun, Y., Kim, S. H., and Kim, K. (2013) Self-crosslinked human serum albumin nanocarriers for systemic delivery of polymerized siRNA to tumors. Biomaterials 34, 9475–9485.
[98] Jonsson, A., Dogan, J., Herne, N., Abrahmsen, L., and Nygren, P. A. (2008) Engineering of a femtomolar affinity binding protein to human serum albumin. Protein Eng. Des. Sel., 21, 515–527.

[99] Huang, M., Han, Y., Zhang, X., Pei, F., Deng, J., Kang, J., and Yan, C. (2010) An intron polymorphism in the CXCL16 gene is associated with increased risk of coronary artery disease in Chinese Han population: a large angiography-based study. Atherosclerosis 210, 160–165.
[100] Ota, K., Quint, P., Weivoda, M. M., Ruan, M., Pederson, L., Westendorf, J. J., Khosla, S., and Oursler, M. J. (2013) Transforming growth factor beta 1 induces CXCL16 and leukemia inhibitory factor expression in osteoclasts to modulate migration of osteoblast progenitors. Bone 57, 68–75.
[101] Ottaviano, G., Cappellesso, R., Mylonakis, I., Lionello, M., Favaretto, N., Giacomelli, L., Spoladore, C., Marchese-Ragona, R., Marino, F., Staffieri, A., Martini, A., and Marioni, G. (2015) Endoglin (CD105) expression in sinonasal polyposis. Eur. Arch. Otorhinolaryngol. 272, 3367–3373.
[102] Marioni, G., D'Alessandro, E., Giacomelli, L., and Staffieri, A. (2010) CD105 is a marker of tumour vasculature and a potential target for the treatment of head and neck squamous cell carcinoma. J. Oral. Pathol. Med. 39, 361–367.
[103] Witkowska, A. M., and Borawska, M. H. (2004) Soluble intercellular adhesion molecule-1 (sICAM-1): an overview. Eur. Cytokine Netw. 15, 91–98.
[104] Ries, C. (2014) Cytokine functions of TIMP-1. Cell Mol. Life Sci. 71, 659–672.
[105] Notohamiprodjo, S., Djafarzadeh, R., Rieth, N., Hofstetter, M., Jaeckel, C., and Nelson, P. J. (2012) Cell surface engineering of renal cell carcinoma with glycosylphosphatidylinositol-anchored TIMP-1 blocks TGF- beta 1 activation and reduces regulatory ID gene expression. Biol. Chem. 393, 1463–1470.
[106] Sufiun, A., Rafiq, K., Fujisawa, Y., Rahman, A., Mori, H., Nakano, D., Kobori, H., Ohmori, K., Masaki, T., Kohno, M., and Nishiyama, A. (2015) Effect of dipeptidyl peptidase-4 inhibition on circadian blood pressure during the development of salt-dependent hypertension in rats. Hypertens. Res. 38, 237–243.
[107] Arno, A. I., Gauglitz, G. G., Barret, J. P., and Jeschke, M. G. (2014) New molecular medicine-based scar management strategies. Burns 40, 539–551.
[108] Siddiqui, S. S., Siddiqui, Z. K., and Malik, A. B. (2004) Albumin endocytosis in endothelial cells induces TGF-beta receptor II signaling. Am. J. Physiol. Lung Cell Mol. Physiol. 286, L1016–L1026.
[109] Elliott, R. L., and Blobe, G. C. (2005) Role of transforming growth factor Beta in human cancer. J. Clin. Oncol. 23, 2078–2093.
[110] Chuang, Y. H., Wang, Y. H., Chang, T. K., Lin, C. J., and Li, P. C. (2014) Albumin acts like transforming growth factor beta1 in microbubble-based drug delivery. Ultrasound Med. Biol. 40, 765–774.
[111] Ribeiro, M., Monteiro, F. J., and Ferraz, M. P. (2012) Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter 2, 176–194.
[112] Ruiz, V., Rodriguez-Cerrato, V., Huelves, L., Del Prado, G., Naves, P., Ponte, C., and Soriano, F. (2011) Adherence of Streptococcus pneumoniae to polystyrene plates and epithelial cells and the antiadhesive potential of albumin and xylitol. Pediatr. Res. 69, 23–27.
[113] Herrmann, M., Vaudaux, P. E., Pittet, D., Auckenthaler, R., Lew, P. D., Schumacher-Perdreau, F., Peters, G., and Waldvogel, F. A. (1988) Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. J. Infect. Dis. 158, 693–701.
[114] Brokke, P., Dankert, J., Carballo, J., and Feijen, J. (1991) Adherence of coagulase-negative staphylococci onto polyethylene catheters in vitro and in vivo: a study on the influence of various plasma proteins. J. Biomater. Appl. 5, 204–226.
[115] Kinnari, T. J., Salonen, E. M., and Jero, J. (2001) New method for coating tympanostomy tubes to prevent tube occlusions. Int. J. Pediatr. Otorhinolaryngol. 58, 107–111.
[116] Kinnari, T. J., Peltonen, L. I., Kuusela, P., Kivilahti, J., Kononen, M., and Jero, J. (2005) Bacterial adherence to titanium surface coated with human serum albumin. Otol. Neurotol. 26, 380–384.
[117] McDowell, S. G., An, Y. H., Draughn, R. A., and Friedman, R. J. (1995) Application of a fluorescent redox dye for enumeration of metabolically active bacteria on albumin-coated titanium surfaces. Lett. Appl. Microbiol. 21, 1–4.
[118] An, Y. H., Stuart, G. W., McDowell, S. J., McDaniel, S. E., Kang, Q., and Friedman, R. J. (1996) Prevention of bacterial adherence to implant surfaces with a crosslinked albumin coating in vitro. J. Orthop. Res. 14, 846–849.
[119] An, Y. H., Bradley, J., Powers, D. L., and Friedman, R. J. (1997) The prevention of prosthetic infection using a cross-linked albumin coating in a rabbit model. J. Bone J. Surg. Br. 79, 816–819.
[120] Naves, P., del Prado, G., Huelves, L., Rodriguez-Cerrato, V., Ruiz, V., Ponte, M. C., and Soriano, F. (2010) Effects of human serum albumin, ibuprofen and N-acetyl-L-cysteine against biofilm formation by pathogenic *Escherichia coli* strains. J. Hosp. Infect. 76, 165–170.
[121] del Prado, G., Ruiz, V., Naves, P., Rodriguez-Cerrato, V., Soriano, F., and del Carmen Ponte, M. (2010) Biofilm formation by Streptococcus pneumoniae strains and effects of human serum albumin, ibuprofen, N-acetyl-l-cysteine, amoxicillin, erythromycin, and levofloxacin. Diagn. Microbiol. Infect. Dis. 67, 311–318.
[122] Horvathy, D. B., Vacz, G., Cselenyak, A., Weszl, M., Kiss, L., and Lacza, Z. (2013) Albumin-coated bioactive suture for cell transplantation. Surg. Innov. 20, 249–255.
[123] Bernards, M. T., Qin, C., and Jiang, S. (2008) MC3T3-E1 cell adhesion to hydroxyapatite with adsorbed bone sialoprotein, bone osteopontin, and bovine serum albumin. Colloid. Surf. B, Biointerfaces 64, 236–247.
[124] Weszl, M., Skaliczki, G., Cselenyak, A., Kiss, L., Major, T., Schandl, K., Bognar, E., Stadler, G., Peterbauer, A., Csonge, L., and Lacza, Z. (2012) Freeze-dried human serum albumin improves the adherence and proliferation of mesenchymal stem cells on mineralized human bone allografts. J. Orthop. Res. 30, 489–496.
[125] Skaliczki, G., Schandl, K., Weszl, M., Major, T., Kovacs, M., Skaliczki, J., Szendroi, M., Dobo-Nagy, C., and Lacza, Z. (2013) Serum albumin enhances bone healing in a nonunion femoral defect model in rats: a computer tomography micromorphometry study. Int. Orthop. 37, 741–745.
[126] Klara, T., Csonge, L., Janositz, G., Csernatony, Z., and Lacza, Z. (2014) Albumin-coated structural lyophilized bone allografts: a clinical report of 10 cases. Cell Tissue Bank. 15, 89–97.
[127] Schandl, K., Horvathy, D. B., Doros, A., Majzik, E., Schwarz, C. M., Csonge, L., Abkarovits, G., Bucsi, L., and Lacza, Z. (2016) Bone-Albumin filling decreases donor site morbidity and enhances bone formation after anterior cruciate ligament reconstruction with bone-patellar tendon-bone autografts. Int Orthop, 40, 2097–2104.
[128] Horvathy, D. B., Vacz, G., Szabo, T., Szigyarto, I. C., Toro, I., Vamos, B., Hornyak, I., Renner, K., Klara, T., Szabo, B. T., Dobo-Nagy, C., Doros, A., and Lacza, Z. (2015) Serum albumin coating of demineralized bone matrix results in stronger new bone formation. J. Biomed. Mater. Res. Part B, Appl. Biomater. 104, 126–132.
[129] Horvathy, D., Vacz, B., Toro, G., Szabo, I., May, T. Z., Duarte, Hornyak, M. I., Szabo, B., Dobo-Nagy, T., Doros, C., and Lacza, A. Z. (2015) Remineralization of demineralized bone matrix in critical size cranial defects in rats: A 6-month follow-up study. J. Biomed. Mater. Res. B Appl. Biomater. 104, 1336–1342.
[130] Liu, X., Zhou, X., Li, S., Lai, R., Zhou, Z., Zhang, Y., and Zhou, L. (2014) Effects of titania nanotubes with or without bovine serum albumin loaded on human gingival fibroblasts. Int. J. Nanomed. 9, 1185–1198.
[131] Nseir, N., Regev, O., Kaully, T., Blumenthal, J., Levenberg, S., and Zussman, E. (2013) Biodegradable scaffold fabricated of electrospun albumin fibers: mechanical and biological characterization. Tissue Eng. Part C Methods 19, 257–264.
[132] Noszczyk, B. H., Kowalczyk, T., Lyzniak, M., Zembrzycki, K., Mikulowski, G., Wysocki, J., Kawiak, J., and Pojda, Z. (2015) Biocompatibility of electrospun human albumin: a pilot study. Biofabrication 7, 015011.
[133] Gallego, L., Junquera, L., Garcia, E., Garcia, V., Alvarez-Viejo, M., Costilla, S., Fresno, M. F., and Meana, A. (2010) Repair of rat mandibular bone defects by alveolar osteoblasts in a novel plasma-derived albumin scaffold. Tissue Eng. Part A 16, 1179–1187.
[134] Gallego, L., Junquera, L., Meana, A., Alvarez-Viejo, M., and Fresno, M. (2010) Ectopic bone formation from mandibular osteoblasts cultured in a novel human serum-derived albumin scaffold. J. Biomater. Appl. 25, 367–381.

[135] Li, P. S., Lee, I. L., Yu, W. L., Sun, J. S., Jane, W. N., and Shen, H. H. (2014) A novel albumin-based tissue scaffold for autogenic tissue engineering applications. Sci. Rep. 4, 5600.
[136] Mueller, B., Koch, D., Lutz, R., Schlegel, K. A., Treccani, L., and Rezwan, K. (2014) A novel one-pot process for near-net-shape fabrication of open-porous resorbable hydroxyapatite/protein composites and in vivo assessment. Mater. Sci. Eng. C Mater. Biol. Appl. 42, 137–145.
[137] Yamaguchi, M., Igarashi, A., Misawa, H., and Tsurusaki, Y. (2003) Enhancement of albumin expression in bone tissues with healing rat fractures. J. Cell. Biochem. 89, 356–363.
[138] Ishida, K., Sawada, N., and Yamaguchi, M. (2004) Expression of albumin in bone tissues and osteoblastic cells: involvement of hormonal regulation. Int. J. Mol. Med. 14, 891–895.
[139] Ishida, K., and Yamaguchi, M. (2004) Role of albumin in osteoblastic cells: enhancement of cell proliferation and suppression of alkaline phosphatase activity. Int. J. Mol. Med. 14, 1077–1081.
[140] Ishida, K., and Yamaguchi, M. (2005) Albumin regulates Runx2 and alpha1 (I) collagen mRNA expression in osteoblastic cells: comparison with insulin-like growth factor-I. Int. J. Mol. Med. 16, 689–694.
[141] Weisiger, R., Gollan, J., and Ockner, R. (1981) Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. Science 211, 1048–1051.
[142] Fasano, M., Fanali, G., Leboffe, L., and Ascenzi, P. (2007) Heme binding to albuminoid proteins is the result of recent evolution. IUBMB Life 59, 436–440.
[143] Fanali, G., di Masi, A., Trezza, V., Marino, M., Fasano, M., and Ascenzi, P. (2012) Human serum albumin: from bench to bedside. Mol. Aspect. Med. 33, 209–290.
[144] van der Vusse, G. J. (2009) Albumin as fatty acid transporter. Drug Metab. Pharmacokinet. 24, 300–307.
[145] Sleep, D., Cameron, J., and Evans, L. R. (2013) Albumin as a versatile platform for drug half-life extension. Biochim. Biophys. Acta 1830, 5526–5534.
[146] Bern, M., Sand, K. M., Nilsen, J., Sandlie, I., and Andersen, J. T. (2015) The role of albumin receptors in regulation of albumin homeostasis: Implications for drug delivery. J. Control Release 211, 144–162.
[147] Kim, J., Bronson, C. L., Hayton, W. L., Radmacher, M. D., Roopenian, D. C., Robinson, J. M., and Anderson, C. L. (2006) Albumin turnover: FcRn-mediated recycling saves as much albumin from degradation as the liver produces. Am. J. Physiol. Gastrointest. Liver Physiol. 290, G352–G360.